

5 October 2012 | \$10

Science

Depression

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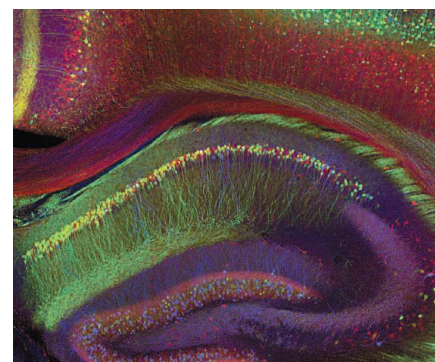
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A solitary figure walks through a somber urban landscape. The permeating tone of sadness in this painting gives an impression of life with major depressive disorder, whereas the light punctuating the scene hints at hope. Because depression has become a serious problem in societies around the world, it is crucial to investigate the basic mechanisms underlying this disease and to develop effective prevention and therapies. See the special section beginning on page 67. For the story behind the cover, go to <http://scim.ag/cov6103>.

Image: "Midnight Stroll" by Michael Bishop, <http://mbishopart.artweb.com/>

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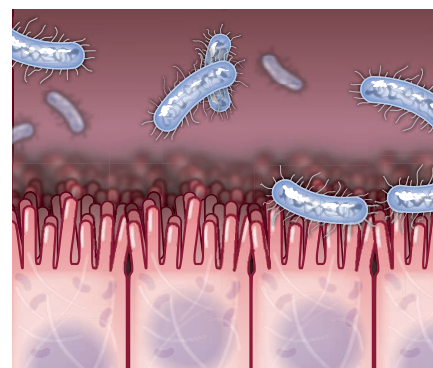
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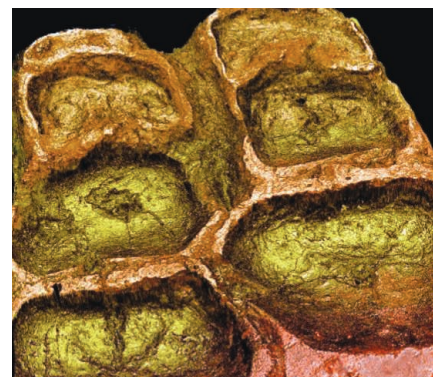
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10.1126/science.1228604

Offspring from Oocytes Derived from in vitro Primordial Germ Cell–like Cells in Mice

K. Hayashi et al.

10.1126/science.1226889

IRE1 α Cleaves Select microRNAs During ER Stress to Derepress Translation of Proapoptotic Caspase-2

J.-P. Upton et al.

10.1126/science.1226191

Elfn1 Regulates Target-Specific Release Probability at CA1-Interneuron Synapses

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10.1126/science.1222482

Intramitochondrial Transport of Phosphatidic Acid in Yeast by a Lipid Transfer Protein

M. Connerth et al.

10.1126/science.1225625

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Highlights From Our Daily News Coverage

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Personality helps forge bonds and may extend survival in baboons.

http://scim.ag/Primates_Survival

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A deadly strain may have ridden the coattails of the AIDS epidemic.

http://scim.ag/Salmonella_Africa

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A toy octopus can teach us about acidification's impact on deep-sea crustaceans.

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SCIENCE SIGNALING

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RESEARCH ARTICLE: The Rho Exchange Factors Vav2 and Vav3 Control a Lung Metastasis-Specific Transcriptional Program in Breast Cancer Cells
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PERSPECTIVE: Rho GEFs and Cancer—

Linking Gene Expression to Metastasis
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Two Vav isoforms could be targeted to prevent breast tumors from metastasizing to the lung.

RESEARCH ARTICLE: An Interaction Between BZR1 and DELLAs Mediates Direct Signaling Crosstalk Between Brassinosteroids and Gibberellins in *Arabidopsis*

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PERSPECTIVE: HCN1 Channels—A New Therapeutic Target for Depressive Disorders?

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The distinct activities of glucocorticoid receptor variants may affect glucocorticoid therapy.

SCIENCE TRANSLATIONAL MEDICINE

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Integrating Medicine and Science

3 October issue: <http://scim.ag/stm100312>

EDITORIAL: The Battlefield of Rare Diseases—Where Uncommon Insights Are Common

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RESEARCH ARTICLE: A Translational Paradigm for the Preclinical Evaluation of the Stroke Neuroprotectant Tat-NR2B9c in Gyrencephalic Nonhuman Primates

D. J. Cook et al.

Primates treated with a neuroprotectant after stroke showed outcomes that mimicked those of a human trial.

RESEARCH ARTICLE: Prevention of Alveolar Destruction and Airspace Enlargement in a Mouse Model of Pulmonary Lymphangioleiomyomatosis (LAM)

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Rapamycin and simvastatin treatment of a mouse model of pulmonary lymphangioleiomyomatosis is described.

RESEARCH ARTICLE: Rapid Whole-Genome Sequencing for Genetic Disease Diagnosis in Neonatal Intensive Care Units

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Rapid whole-genome sequencing of neonates can shorten time to disease diagnosis and counseling.



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COMMENTARY: “What Great Creation”

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SCIENCE CAREERS

www.sciencereers.org/career_magazine

Free Career Resources for Scientists

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Gauging Graduates' Gloom

M. Price

Graduate students' pressures make them especially prone to depression, but small changes can help.

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Taken for Granted: America's Real Jobs Gap

B. L. Benderly

A new book solves the mystery of America's employment situation and tells how to fix it.

A Career for Two, With Empathy

E. Pain

A husband-and-wife team studies the brain areas that allow us to feel what others feel.

SCIENCE PODCAST

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Free Weekly Show for 5 October 2012

Depression resilience, properties of dinosaur teeth, mysteries of the brain, and more.

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ADVANCING SCIENCE. SERVING SOCIETY

Plant Anti-Insect Armaments >>

Because individual plants are unable to relocate, they are subject to extreme selection by the insects feeding upon them. One means by which plants suppress herbivory is to produce toxic compounds to deter feeding (see the Perspective by Hare). Agrawal *et al.* (p. 113) compared pesticide-treated or untreated evening primroses. Over 5 years of pesticide treatment, the production of defensive chemicals in the fruit reduced and flowering times shifted, and the primrose's competitive ability against dandelions improved. Züst *et al.* (p. 116) examined large-scale geographic patterns in a polymorphic chemical defense locus in the model plant *Arabidopsis thaliana* and found that it is matched by changes in the relative abundance of two specialist aphids. Thus, herbivory has strong and immediate effects on the local genotypic composition of plants and traits associated with herbivore resistance.

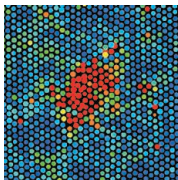


Grinding Out Earthquake Physics

Simulating earthquakes in the lab produces events that are often orders of magnitude lower in energy than large, damaging earthquakes; thus, it is unclear how well the behavior along these simulated faults extrapolates to the physics of large earthquakes. Chang *et al.* (p. 101; see the Perspective by Shimamoto and Togo) used a rotary apparatus that spins in tandem at high speeds with a disc-shaped rock sample, producing energies comparable to earthquakes of magnitudes 4 to 8. Experiments using both granite and dolomite samples suggest that the weakening of slip along faults during large earthquakes is a product of intense accelerations associated with the rupture event.

Homogeneous Melting

The nucleation and melting of crystals are primarily driven by surfaces and defects, which can lower the thermodynamic barrier to a phase transition. A harder problem to study is when the transition occurs uniformly. Wang *et al.* (p. 87; see the Perspective by Weeks) imaged the homogeneous melting of superheated colloidal crystals using a laser to initiate the melting at the interior of the crystal. The authors were then able to track nucleation precursors and nucleus evolution and to find where defects and instabilities limited the homogeneous melting process.



Watching Supercoiled DNA

The DNA double helix can undergo additional twisting, or supercoiling, that plays a role in transcription and protein binding, in part by

bringing distant DNA locations together. The process forms intertwined loops, called plectonemes, and van Loenhout *et al.* (p. 94, published online 13 September; see the Perspective by Sheinin and Wang) visualized plectoneme dynamics of fluorescently labeled, 21-kilobase tethered DNA molecules using magnetic tweezers to apply twisting forces. Plectonemes could diffuse along the DNA, but move more rapidly if they "hopped"—nucleating a plectoneme at a new position.

Theory Drives Understanding

The role of theory and simulation in neuroscience has been hotly debated over the past few years, in particular in the context of the recent launch of several big projects aimed at creating artificial or virtual brains. Gerstner *et al.* (p. 60) review how theory and simulations have interacted over the years and how they have contributed to our present view of how the brain works.

Reversing Autism in Mice

Autism comprises a heterogeneous group of neurodevelopmental disorders characterized by defects in communication and social interaction. A group of nonsyndromic forms of autism is associated with mutations in the neuroligin genes, which encode postsynaptic adhesion molecules. Using a reversible knockout approach, Baudouin *et al.* (p. 128, published online 13 September) investigated the in vivo functions of neuroligin-3 in the mouse cerebellum. Mutant mice showed a major defect in metabotropic glutamate receptor-dependent, long-term potentiation; disrupted heterosynaptic competition; and ectopic synapse formation in vivo. These synaptic defects could be rescued by reactivation of the neuroligin gene in the adult.

Lending a Hand to CO₂ Reduction

Although plants and microbes have been reducing CO₂ with ease for millennia, people still find it extremely challenging. A cost-effective synthetic scheme for transforming CO₂ into fuels and commodity chemicals would be a double boon, lowering atmospheric concentration of the greenhouse gas while supplementing (and perhaps ultimately replacing) dwindling petroleum feedstocks. Toward this end, Costentin *et al.* (p. 90) show that an iron catalyst for the electrochemical reduction of CO₂ to CO gets an efficiency boost from phenol substituents appended to the ligand framework.

A Toothy Problem

Large mammalian herbivores such as horses and bison are well known to possess a complex, grinding dentition that facilitates processing of their tough, cellulose-rich plant diet. Hadrosaurid, or duck-billed, dinosaurs also possessed complex teeth, but how this was achieved has been unknown because reptiles typically possess simple teeth. Erickson *et al.* (p. 98) show how Hadrosaurs evolved teeth composed of six tissues, which allowed for the development of tooth complexity rivaling, or exceeding, that of modern herbivorous mammals.

Puzzling Through Gravity

Much of the excitement of scientific discovery seems to get lost when science is taught as facts by lectures. Granger *et al.* (p. 105) present a large study of outcomes comparing inquiry-based teaching with more traditional teaching methods. Over 2000 students were involved, in 125 classrooms of 4th- and 5th-graders. The

Continued on page 13

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classes studied space-science with a curriculum that uses models and evidence to entice students into improving their own understanding of the science. Students who were encouraged to use evidence to support their models seemed to develop improved knowledge of content.

Of Microbes and Cancer

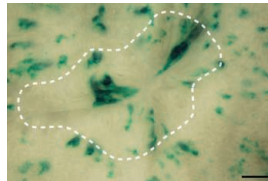
Inflammation is a well-established driver of tumorigenesis. For example, patients with inflammatory bowel disease have an elevated risk of developing colorectal cancer (CRC). Whether the gut microbiota also contributes to the development of CRC is less well understood. **Arthur *et al.*** (p. 120, published online 16 August; see the Perspective by **Schwabe and Wang**) now show that the microbiota does indeed promote tumorigenesis in an inflammation-driven model of CRC in mice. Although germ-free mice were protected against developing cancer, colonization of mice with *Escherichia coli* was sufficient to drive tumorigenesis.

Regulating Opioid Responses

Different drugs of abuse are thought to hijack similar reward systems in the brain using common mechanisms. However, **Koo *et al.*** (p. 124) now observe that some of the neural mechanisms that regulate opiate reward can be both different and even opposite to those that regulate reward by stimulant drugs. While knockdown of brain-derived neurotrophic factor (BDNF) in the ventral tegmental area in mice antagonized the response to cocaine, the same manipulation strengthened the potential of opiates to increase dopamine neuron excitability. Optogenetic stimulation of dopaminergic terminals in the nucleus accumbens could counteract the effects of BDNF on morphine reward blockade.

Gut, Heal Thyself

Foods, drugs, and pathogens all represent possible threats to our guts on a daily basis. Fortunately, the gut is quite good at repairing itself—but how? Working in mice, **Miyoshi *et al.*** (p. 108, published online 6 September; see the Perspective by **Barrett**) selectively injured intestinal crypts containing intestinal stem cells and observed the repair process. The noncanonical Wnt ligand, Wnt5a, was required for crypt regeneration. Wnt5a inhibited intestinal stem cell proliferation, which paradoxically promoted regeneration of crypt tissue.



Salience, Values, and Decisions

How does the brain make value-based decisions? There are two major competing models: the goods-based model and the action-based model of value. **Leathers and Olson** (p. 132) designed a critical experiment to decide between these two views. In the monkey brain, lateral intraparietal neurons responded strongly to stimuli predicting both large rewards and large penalties, encoding the salience of the stimulus rather than reward value, which refutes both models.

Changing Your Belief

The ability to display behavioral flexibility depends on an internal representation of the environment—a framework of beliefs that can be adjusted on the basis of experience. Recording from multiple electrodes in the rat medial prefrontal cortex, **Karlsson *et al.*** (p. 135) investigated how ensembles of neurons changed their activity during the performance of a task in which the animal had to update its knowledge of reward contingencies. The results suggest that changes in perceived action-outcome contingencies were associated with abrupt switches in neuronal representations in the rat medial prefrontal cortex.

Close to a Black Hole

At the center of our Galaxy, there is a black hole that is 4 million times as massive as the Sun. Using data from the Keck Observatory, **Meyer *et al.*** (p. 84) detected a star orbiting this black hole with a period of 11.5 years, the shortest period among the stars orbiting it. The star is the second well-sampled star with an orbital period under 20 years. Having detailed knowledge about two stars with short periods and full orbit coverage will be crucial in testing Einstein's theory of general relativity in the gravitational field close to a massive black hole.

CREDIT: MIYOSHI ET AL.



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The Burden of Mood Disorders

THE OVERALL BURDEN OF MOOD DISORDERS—INDIVIDUAL, SOCIETAL, AND ECONOMIC—HAS BEEN increasing in recent decades. This is certainly the case for depression (see the special section beginning on page 67 of this issue). In Europe, the burden is greater than 10 years ago despite the availability of reasonably effective pharmacological and psychological interventions.* Yet the prevalence of mood disorders has remained steady at approximately 10% of the population.*† What explains the increased cost and societal burden of depression and bipolar disorders, and what can be done to improve the situation?

The major barrier to improving treatment in most countries has been limited financial and structural resources. A 2011 European Brain Council Report‡ revealed that unlike cardiac disease and diabetes, in which direct costs (diagnosis and treatment) typically account for over 50% of the total cost, mood disorders are dominated by indirect costs such as sick days, unemployment, long-term disability, and suicide attempts (direct costs account for about 23% of the total). A second factor, which is beyond the control of medical professionals, is the consequence of longer life spans. Living longer is associated with an increased probability of developing depression with a more malignant course due to, for example, vulnerability and risk factors that arise from other chronic conditions.*

There has also been a failure to generate the needed multidisciplinary models of disease intervention and effective mental health services. Mood disorders are highly complicated, with neurobiological, behavioral, and psychological etiologic pathways, and there is a range of complex treatment goals. Interventions also need to be tailored to address multiple factors, including the type of mood disorder, its severity, comorbid complications, and the patient's preference for treatment. Current models of pharmacological or psychological interventions and even personalized medicine approaches fail to account for these issues sufficiently, in part because of a lack of knowledge regarding the neural underpinnings of the highly dynamic pathological pathways. Given that most patients are not recognized in a timely manner by physicians, never seen by a specialist, or not treated appropriately according to minimal guideline standards, the increase in the overall burden of mood disorders is not surprising.

Improved integrative models, based on research in the behavioral, social, and neurosciences, are urgently needed that better reflect the heterogeneity and complexity of mood disorders. The goal is to provide a range of effective interventions within a personalized perspective, taking into account the vulnerability and risk factors responsible for one's clinical trajectory to illness, its duration, and remission, in addition to pharmacogenomic and other biomarker approaches. Early interventions based on personalized approaches have the most promise.

Two-thirds of mood disorders start in late adolescence and early adulthood, predominantly as secondary conditions to other types of preexisting and untreated mental disorders, such as anxiety, attention deficit disorder, and addiction. These preceding disorders are powerful risk factors for the first onset of a mood disorder, and they can predict a malignant and chronic course as well as a poorer response to treatment. Early intervention targeted to such highly vulnerable patients can decrease not only the incidence of mood disorders but also the degree of disability and depression burden.§

Reducing the burdens of mood disorders requires major shifts in research, clinical practice, and public health by incorporating multidisciplinary models of intervention. The good news is that such changes are under way, as reflected, for example by the experts drafting Research Roadmaps (see www.roamer-mh.org) for the European Union.

— Hans-Ulrich Wittchen

10.1126/science.1230817

*H.-U. Wittchen *et al.*, *Eur. Neuropsychopharmacol.* **21**, 655 (2011). †E. Bromet *et al.*, *BMC Med.* **9**, 90 (2011).

‡A. Gustavsson *et al.*, *Eur. Neuropsychopharmacol.* **21**, 718 (2011). §H.-U. Wittchen *et al.*, *Eur. Psych.* **18**, 384 (2003).



GENETICS

Understanding Recombination

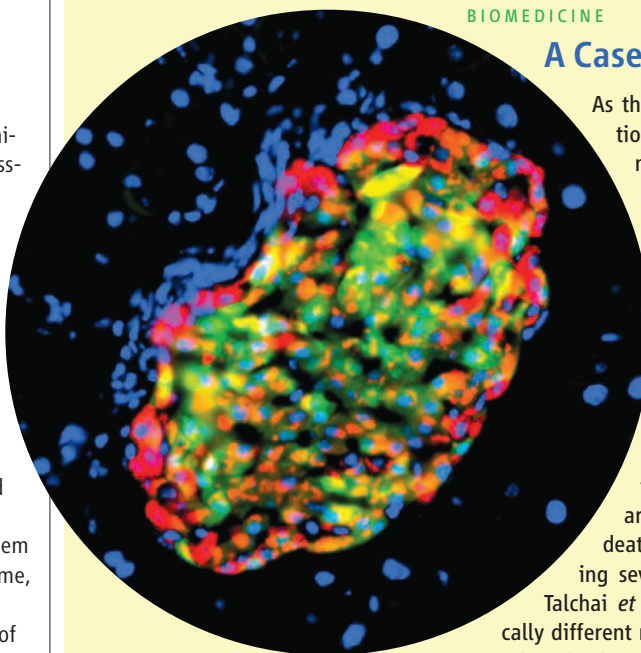
Recombination, the process of chromosomal exchanges to foster genetic variation, occurs variably along the genomes of plants and animals, with certain regions showing more crossovers than others. One feature that controls crossovers is the presence or absence of methylation on the DNA sequences, where densely methylated regions show low rates of crossover events. Colomé-Tatché *et al.* examined genome-wide recombination patterns in *Arabidopsis* plants with parents of differing methylation status: one heavily methylated and the other hypomethylated. Among the offspring, regions of high and low methylation were traced and genome-wide recombination events were inferred. Although methylation patterns did seem to affect crossover rates in parts of the genome, crossovers were suppressed near the centromere, even in individuals with low amounts of methylation in this area. These results suggest that recombination events in this region are insensitive to natural DNA sequence variation and methylation state and help to provide a framework to delineate the epigenetic basis of complex traits in *Arabidopsis*. — LMZ

Proc. Natl. Acad. Sci. U.S.A. **109**, 10.1073/pnas.1212955109 (2012).

MATERIALS SCIENCE

Ising Ice

Spin ice, a group of exotic magnetic materials that have molecular arrangements akin to that of water ice, can be artificially engineered out of nanomagnets arranged in patterns that resemble real crystal lattices, but with fully tunable properties. Normally, these nanomagnets orient themselves within the plane that they occupy; Zhang *et al.* created an artificial spin ice in which the magnetic moments point perpendicular to the plane—a realization of the canonical Ising spin model. Magnetic force microscopy confirmed that, after initialization, the magnets pointed either up or down. The frustrated kagome lattice, with perpendicular magnetization, showed similar spatial correlation properties to those of the honeycomb lattice, with in-plane moments, implying that the behavior is dominated by the nearest-neighbor interactions, which are matched between the two; the similarity was maintained through a range of interactions. The realization of perpendicular magnetization is expected to lead to interesting hybrid systems with thin films, in which the properties of the adjacent films may



BIOMEDICINE

A Case of Lost Identity?

As the cells responsible for the secretion of insulin—the major hormonal regulator of blood glucose levels—pancreatic β cells are the central command center for the prevention of type 2 diabetes. When β cells cannot keep pace with metabolic demands (which occurs with obesity, during pregnancy, and as people age), insulin secretion drops and blood glucose levels rise. The prevailing model is that β cells fail because they are lost through programmed cell death and are not replaced. Studying several mouse models of diabetes, Talchai *et al.* present evidence for a radically different mechanism of β cell failure: They propose that the loss of β cells occurs because under conditions of physiological stress, the cells change their identity through de-differentiation to a more progenitor-like state and are then converted to other pancreatic cell types that do not secrete insulin, such as α cells. The transcription factor FoxO1 plays an essential role in keeping β cells in their differentiated state. If these observations apply to humans, they would suggest that therapies for type 2 diabetes should focus on reversing the β cell de-differentiation process rather than promoting β cell replication. — PAK

Cell **150**, 1223 (2012).

be controllable by the perpendicular magnetic moments. — JS

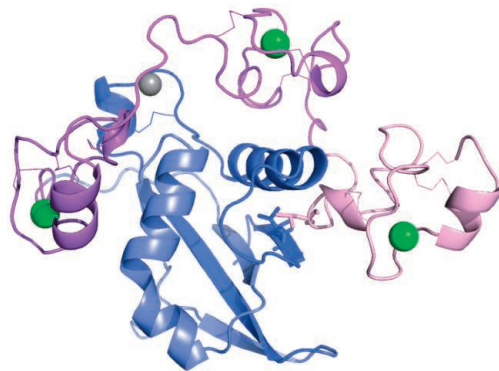
Phys. Rev. Lett. **109**, 087201 (2012).

BIOCHEMISTRY

A Forced Opening

Notch proteins are transmembrane receptors that have major roles in the regulation of cell proliferation and development, and mutations in the Notch signaling pathway contribute to human diseases and cancer. When Notch proteins are bound by their ligands, which are themselves transmembrane proteins found on adjacent cells, endocytosis of the Notch extracellular domain into the ligand-bearing cell occurs, during which the Notch protein is cleaved, allowing the intracellular domain to be translocated to the nucleus, where it activates target genes. Stephenson and Avis used atomic force microscopy (AFM) to physically stretch a portion of the receptor called the Notch negative regulatory region, which contains the masked site of proteolysis. They could show through AFM measurements and molecular dynamics simulations that such a mechanical stimulus unfolded

the protein to expose a site where proteolytic cleavage occurred (and actually monitored the latter as release of the AFM tip). The events and forces measured were consistent with a favored model of Notch signaling in which forces of



ligand endocytosis expose the otherwise buried cleavage site, leading to slicing of the receptor and initiation of signaling. — LBR

Proc. Natl. Acad. Sci. U.S.A. **109**, 10.1073/pnas.1205788109 (2012).

Continued on page 19

Continued from page 17

ENVIRONMENTAL SCIENCE

A Sea of Difference

Large-scale anthropogenic impacts on the environment are often considered to be products of modern agricultural and industrial activity. It is becoming clearer, however, that humans have been agents of widespread environmental change for much longer than that. Through a combination of sedimentary, paleoenvironmental, and paleogenetic evidence, Giosan *et al.* chronicle how the biogeochemistry of the Black Sea has changed over the past 7500 years and how humans contributed to that. Surface salinity decreased and the delta of the Danube expanded over the past 2000 years, as sediments delivered by the Danube, the main tributary of the Black Sea, increased in volume because of the intensification of land use throughout its watershed. Over the past five to six centuries, greater nutrient fluxes resulting from rapid deforestation in Eastern Europe caused the proliferation of diatoms and dinoflagellates and

onation steps, which taken together with prior studies establish that the water-splitting cycle proceeds through strictly alternating extraction of electrons and protons.

Many synthetic systems focus on forming hydrogen from water-derived protons, rather than replicating the natural CO₂ functionalization cycle. Marinescu *et al.* probed the mechanism for one such catalyst that relies on redox-active cobalt. Using nuclear magnetic resonance spectroscopy, they tracked the rate of disappearance of a Co(III)-H intermediate, uncovering a bimolecular decay inhibited by increasing proton (acid) concentration. Analysis of these results together with cyclic voltammetry data and numerical simulations showed that the dominant mechanism involved a one-electron reduction of the intermediate by unprotonated Co(I) before H₂ formation, with a slower concurrent direct reaction involving two Co(III)-H complexes. — JSY

Proc. Natl. Acad. Sci. U.S.A. **109**, 10.1073/pnas.1206266109; 10.1073/pnas.1213442109 (2012).

IMMUNOLOGY

A Strategic Defense

Just as in American football, during the immune response, the location of your defenders is key. One player out of line can make the difference between a sack or a touchdown, or in the case of the immune system, a localized versus systemic infection. How the immune system orchestrates this careful defense, however, is not well understood. Kastenmüller *et al.* now demonstrate that the organization of cells within the lymph nodes of mice is critical for preventing pathogen spread during

the first few hours of an infection. Infecting bacteria drain to nearby lymph nodes, where they are immediately collected by a specially localized population of macrophages. Although these macrophages have antimicrobial activity, the immune system takes further precautions to ensure that the infection does not spread. Several types of lymphoid cells are localized near these macrophages. In response to bacteria, an inflammasome-dependent mechanism drove macrophages to produce the cytokine interleukin-18, which in turn alerted the nearby lymphoid cells to the threat. In response, the lymphoid cells produced the cytokine interferon- γ , which enhanced the antimicrobial activity of the macrophages, thus keeping the infection in check. — KLM

Cell **150**, 1235 (2010).



changed the entire food web structure of the Black Sea. Thus, long before industrialization, the Black Sea was much different from the time before humans populated its shores. — HJS

Sci. Rep. **2**, 10.1038/srep00582 (2012).

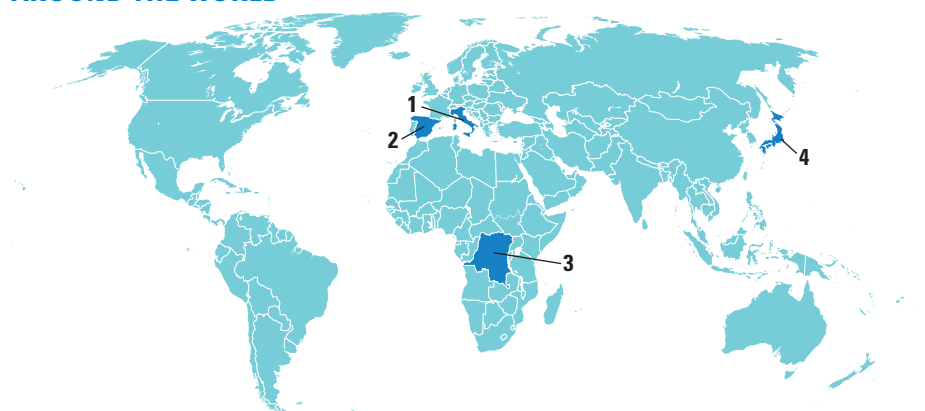
CHEMISTRY

Protons Coming and Going

Photosynthesis channels light into chemical energy by splitting water. Klauss *et al.* explored the sequence of steps whereby the manganese cluster in plants and algae extracts four protons and four electrons from two water molecules, forming O₂ in the process. By applying a photothermal beam deflection technique to probe the relative timing of electron and proton transfers, the authors uncovered two deprotonation

CREDIT: NASA

AROUND THE WORLD



L'Aquila, Italy 1

Prison Terms Sought for Italian Earthquake Experts

Seven experts tapped for advice before a deadly earthquake struck L'Aquila, Italy, in April 2009 should each get 4 years in jail, prosecutors in their manslaughter trial in the central Italian city requested on 25 September.

The four scientists, two engineers, and a public official are accused of conducting a superficial risk analysis that gave townsfolk a false sense of security before the quake. The seven—members of Italy's National Commission for the Forecast and Prevention of Major Risks—had met on 31 March 2009 to assess the danger posed by seismic tremors that had been shaking L'Aquila and the surrounding area for 3 months.



A draft version of the commission's meeting minutes contains controversial statements absent in the official minutes, including references to the discredited idea that small tremors reduce the chances of a bigger quake because they discharge energy. Victims' relatives said that misconception persuaded loved ones to stay indoors before the quake hit.

Judge Marco Billi is due to reach a verdict by 23 October. <http://scim.ag/EQtrial2012>

Madrid 2

Billions in Spanish Research Budget Unspent

Research and development in Spain will suffer another 7.2% cut if Parliament approves the government's 2013 budget, presented on 29 September. The budget has shrunk 4 years in a row, overall by 38.7% since 2009. But an analysis by the Confederation of Spanish Scientific Societies (COSCE), released on 27 September, shows that the real picture is even bleaker than official numbers suggest because billions in research money simply remains unspent.

Included in the science budget is a pot of money aimed at supporting companies, universities, and public research institutions with loans. In practice, the loans—which made up 58.8% of the 2012 budget—aren't much in demand, and most of the money flows back into state coffers. In 2011, at least €3 billion remained unused; in 2010, €2.36 billion.

COSCE called on the government to look closer at which funding programs are needed and to help ensure companies make use of the loans. Future science budgets should be “in line with the reality of the country,” says COSCE President Carlos Andradás Heranz, a mathematician at the Complutense University of Madrid. <http://scim.ag/Spainbudget>

Democratic Republic of the Congo 3

Ebola-Like Virus Found

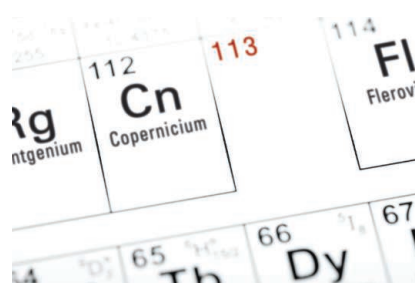
A newly discovered virus from the family that causes rabies may be responsible for three linked cases of hemorrhagic fever in the Democratic Republic of the Congo in 2009. An international team of researchers reported on 27 September in *PLoS Pathogens* the first evidence that a virus from the rhabdovirus family, which typically causes

brain swelling or flulike disease, is a hemorrhagic fever agent like Ebola virus and Marburg virus—among the most virulent pathogens known to infect humans.

Using sophisticated RNA sequencing technology, the researchers discovered a link between a rhabdovirus they called Bas-Congo and a 2009 episode of acute hemorrhagic fever in three people from the village of Mangala. The scientists did not actually isolate Bas-Congo virus, but instead plucked out RNA sequences from a blood sample taken from the outbreak's sole survivor and reconstructed its genome.

“It looks fairly solid,” says Thomas Ksiazek, a hemorrhagic disease specialist at the University of Texas Medical Branch in Galveston. Ksiazek cautions that until you have the actual virus in hand, it's difficult to know what caused the disease.

The research team is now intensifying its hunt for the virus itself in humans and other species. <http://scim.ag/BasCongo>



Wako, Japan 4

Element 113 Clinched?

Researchers in Russia and Japan have sparred for nearly a decade over who first observed an atom of superheavy element 113. Now, the Japanese team from the RIKEN Nishina Center for Accelerator-Based Science in Wako says a new atom of 113—its third—should cement its claim.

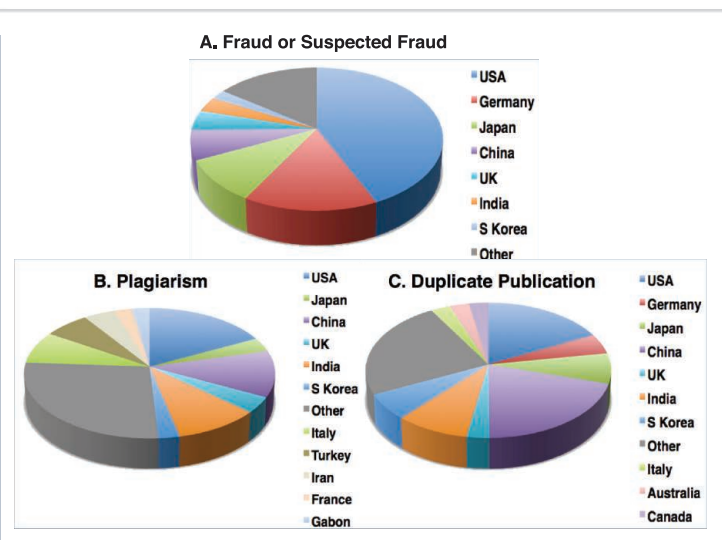
Researchers make superheavy elements by firing nuclei at foil made of a heavy element, hoping that a projectile fuses with a target nucleus. The resulting compound nucleus lasts a fraction of a second and is identified by the chain of unstable isotopes it decays into. Last year, an international adjudicating panel contended that none of the isotopes the groups cited as evidence had been studied, so their identity—and credit for the discovery—remained unclear.

The new RIKEN chain, however, took a different decay route leading to isotopes that had been studied. The result makes a “very strong case” for discovery, says

Most Papers Retracted for Misconduct

Geography helps tell the story of why biomedical papers are retracted—or so suggests a new study published online on 1 October in the *Proceedings of the National Academies of Sciences (PNAS)*. Microbiologists Ferric Fang and Arturo Casadevall, along with medical writer R. Grant Steen, examined the reasons for retractions from multiple angles, and they found that the explanations partly track with the country where the research was done. Most papers retracted for fraud or suspected fraud involved work conducted in the United States. But when plagiarism and duplicate publications are the reason for retractions, locales such as China take a bigger slice of the pie.

More broadly, the *PNAS* study finds that misconduct (which includes fraud, plagiarism, and duplicate publication) accounts for about two-thirds of all retractions, to the authors' surprise. "I thought it was going to be error" that explained why most papers were pulled, says Casadevall of Albert Einstein College of Medicine in the Bronx, New York.



Science has the dubious distinction of coming first in a long list of journals with the most retracted articles in the past 40 years, with 70 retractions, edging out *PNAS* by one. <http://scim.ag/retractstudy>

Christoph Düllmann of the GSI nuclear research lab in Darmstadt, Germany. Will the adjudicators agree? Stay tuned. <http://scim.ag/element113>

NEWSMAKERS

Early Leader of Environmental Movement Dies



Barry Commoner, a cell biologist who became an influential proponent of the 1963 Nuclear Test Ban Treaty, a leader of the burgeoning environmental movement, and a candidate for president, died on 30 September in

New York at the age of 95. Known as an often provocative scholar, Commoner prompted a flurry of letters to *Science* in 1961 with an essay, "In Defense of Biology," that decried environmental threats and bemoaned "a widening gap between the more traditional areas of biology and those which are closely related to modern chemistry and physics" (*Science*, 2 June 1961, p. 1745). In 1970, *Time* magazine put Commoner on its cover, hailing him as a luminary in the "emerging science of survival." But when asked in 1976 what he would do if appointed White House science adviser, Commoner told *Science* he'd resign: "I don't believe in science advice. ... Pressure from an informed public is far better than an advisory system" (*Science*, 6 August 1976, p. 464).

Polar Bear Biologist Cleared Of Misconduct Charges

Charles Monnett, a prominent U.S. government polar bear researcher who had been suspended amid allegations of research misconduct (*Science*, 5 August 2011, p. 681), has returned to work after being cleared of the most serious charges. But he is being reprimanded by his employer, the U.S. Bureau of Ocean Energy Management, Regulation and Enforcement, for e-mailing internal agency documents to several political officials and academics in Alaska. The decision ends a controversy that began in March 2010 when an anonymous government employee complained that Monnett had released information and manipulated data on polar bear mortality to thwart plans by the Shell energy company to drill exploratory wells in the Arctic. Monnett's allies cheered the decision. "We are pleased this misguided witch hunt is finally stumbling to a conclusion," said Jeff Ruch, executive director of Public Employees for Environmental Responsibility in Washington, D.C., which helped defend Monnett against the charges.

Gran Sasso Gets New Director

Physicist **Stefano Ragazzi**, of the University of Milano-Bicocca in Italy, has been appointed the next director of the Gran Sasso National Laboratory in L'Aquila, Italy, run by the Italian National Institute of Nuclear Physics. He replaces physicist Lucia Votano, who

has held the post since 2009, on 15 October. Ragazzi, 57, began his scientific career studying neutrinos and later moved into high-



energy physics. Starting in 1992, he coordinated several high-energy research groups at CERN, the European particle physics lab in Geneva, Switzerland. He also helped design calorimeters—devices

that measure the energy of particles—that CERN's CMS experiment used in the hunt for the Higgs boson. "As a new director, the major challenge will be keeping the level of research as high as it has been so far, especially in the field of dark matter and the study of neutrinos' properties," Ragazzi says.

FINDINGS

Obesity Hormone in Fruit Flies

When it comes to mealtime, fruit flies are more like people than we thought. The insects, researchers have found, churn >>



Random Sample

Alfred Russel Wallace Goes Online



The eminent 19th century British naturalist Alfred Russel Wallace now has his own Web site. Wallace Online (<http://wallace-online.org/>) provides free searchable access to all 28,000 pages of his writings and other historical documents and to 22,000 images. Wallace and Charles Darwin were contemporaries who independently formulated the theory of evolution by natural selection. Aside from Darwin, "Wallace is the only other person in history who independently figured out the secret of life," says John van Wyhe, a science historian at the National University of Singapore who put the treasure trove of information together. Van Wyhe also created the popular Darwin Online Web site (<http://darwin-online.org.uk/>). The new site, timed to mark the hundredth anniversary of the naturalist's death in 1913, includes the full text of Wallace's magnum opus, *Darwinism*, which van Wyhe calls "next to *The Origin of Species*, the single greatest book ever written on evolution."



>>FINDINGS

out the hormone leptin—the same hormone that helps control appetite and metabolism in humans. Leptin has long fascinated scientists, who study it to sort out the molecular underpinnings of obesity. But until now, they thought that only vertebrates produced it. This new find could make it easier to uncover leptin's secrets.

Akhila Rajan, a postdoctoral fellow at Harvard Medical School in Boston, and her adviser, Norbert Perrimon, made the discovery after engineering flies that lacked a nutrient-sensing protein called Upd2. Flies without Upd2 look metabolically as if they're famished. But when Rajan inserted

the human leptin gene into her flies, they didn't have that problem. The researchers concluded in a study published last week in *Cell* that Upd2 is the "functional homolog" of leptin, meaning the protein in flies acts very much as leptin does in people. Because flies are relatively easy to study in the lab, the researchers hope their discovery will make it easier to study leptin's biology. <http://scim.ag/obesefly>

Concrete Evidence for Water On Ancient Mars

Billions of years ago, enough water flowed into Gale crater fast enough to carry gravel to the middle of the crater floor—where the Curiosity rover has found and imaged it, the NASA mission's team reported in a press conference on 27 September. The broken face of a 15-centimeter-thick layer of rock sports bits of stone worn into pebbly roundness as they tumbled down from the nearby



crater rim in a torrent of water. Previous Spirit and Opportunity rover investigations and orbital imaging have found evidence of salty ground water, evanescent puddles of brine, and flowing rivers on ancient Mars. But properties of the newly discovered rock, such as the size range of the gravel, will let researchers infer a great deal more about ancient water on Mars. Unfortunately, gravel laid down in torrential flows is about the worst sort of deposit to search for traces of ancient life. As demonstrated on Earth, the organic remains of long-ago life are far better preserved in the muddy deposits of quiescent lake bottoms. <http://scim.ag/Marswater>

Oocytes Created in a Dish Produce Normal Mice

A Kyoto University team led by stem cell biologist Mitunori Saitou has produced normal mouse pups using oocytes, or immature egg cells, created in vitro from embryonic stem cells and induced pluripotent stem cells (ES cells and iPS cells). The achievement is a first for mammals.

The researchers cultured mouse ES and iPS cells in protein cocktails to produce primordial germ cell-like cells. To get oocytes, they mixed these cells with fetal ovarian cells, forming reconstituted ovaries that they grafted onto natural ovaries in female mice. Four weeks later, the primordial germ cell-like cells had developed into oocytes. After in vitro fertilization, the researchers implanted the resulting embryos into surrogate mothers, producing normal mouse pups, the team reported online on 4 October in *Science*.

"It is remarkable that one can produce oocytes capable of sustaining complete development starting with embryonic stem cells," says developmental biologist Davor Solter of Singapore's Institute of Medical Biology who was not involved with the research. Saitou says that with more work, the team may be able to eliminate the grafting step, generating viable oocytes completely in vitro. In addition to shedding light on early developmental processes, the technique could lead to new human fertility treatments if technical challenges and ethical issues can be resolved, he says. <http://scim.ag/oocytes>

BY THE NUMBERS

\$1.2 trillion Estimated annual worldwide cost of climate change, equivalent to about 1.6% of global GDP, according to a report released on 26 September by DARA and the Climate Vulnerable Forum.

50% Average decline in coral cover on the Great Barrier Reef from 1985 to 2012, according to a study published on 1 October in the *Proceedings of the National Academy of Sciences*.

Science LIVE

Join us on Thursday, 11 October, at 3 p.m. EDT for a live chat on a **hot topic in science**. <http://scim.ag/science-live>

CONDENSED-MATTER PHYSICS

Supersolidity Shot Down By Its Own Discoverer

The complicated mystery of supersolidity appears to be coming to an end, and it's not the thrilling climax that many physicists had hoped for. It now appears that the bizarre phenomenon—in which ultracold, highly pressurized solid helium supposedly flows through itself like a liquid without any viscosity—simply doesn't exist, physicists say. Instead, nearly all the supposed signs of supersolidity seem to have a more mundane explanation.

"I can't be 100% sure that there is no supersolidity, but the evidence is getting weaker and weaker," says Sébastien Balibar, an experimenter at the École Normale Supérieure (ENS) in Paris, who had argued that the phenomenon is real. "Many of us don't believe in it any longer." Anthony Leggett, a theorist at the University of Illinois, Urbana-Champaign, says: "It looks almost like a conspiracy of nature to fool us on this one. The original evidence really looked pretty strong."

Ironically, the data that may mark the end of the story come from the same physicist who reported the first signs of the phenomenon, Moses Chan of Pennsylvania State University, University Park. "I'm hammering the nail in my own coffin," Chan says.

Chan and Eunseong Kim, now at the Korea Advanced Institute of Science and Technology in Daejeon, reported signs of supersolidity in 2004 in *Nature* (*Science*, 1 July 2005, p. 38). The two fashioned a small metal can containing a cylinder of a porous glass called Vycor. They filled the nanometer-sized pores with frigid liquid helium and pressurized the can to more than 25 times atmospheric pressure to solidify the helium. Chan and Kim set the can twisting back and forth atop a thin metal shaft.

The researchers then cooled the twisting "torsional oscillator." As the temperature fell below about 0.2 kelvin, the frequency of twisting suddenly shot up. That suggested that some of the helium atoms were letting go of the can and standing stock-still, effectively making the can lighter and reducing its inertia. To do that, the atoms would have to flow through the rest of the helium without any resistance.

That notion may sound fanciful, but



"I'm hammering the nail in my own coffin."

—MOSES CHAN, PENNSYLVANIA STATE UNIVERSITY, UNIVERSITY PARK

decades earlier, physicists had used the same technique to prove that liquid helium can flow without resistance, a quantum-mechanical phenomenon known as superfluidity. Some theorists had predicted something similar might happen in solid helium. Kim and Chan reported signs of flow in pure solid helium in 2004 in a subsequent paper in *Science*.

Those results were controversial from the start. Perfect crystalline helium shouldn't flow, some theorists argued, and experiments soon showed that the supposed flow appeared only in crystals riddled with atomic-scale defects.

Then John Beamish and James Day of the University of Alberta in Edmonton, Canada, used a different technique to show that solid helium stiffens below 0.2 kelvin, as they reported in *Nature* in 2007. Such stiffening should also cause the frequency of a torsional

oscillator to increase just as flow would. At the time, however, simulations suggested that the stiffening alone could not explain away the observed frequency shifts. So many physicists reasoned that stiffening and flow were parallel effects.

However, recent data have suggested that stiffening may be the *only* effect. Earlier this year, John Reppy, a physicist at Cornell University, performed an experiment on a Vycor-filled "double oscillator" that could twist at two frequencies (*Science*, 11 May, p. 661). If a fixed fraction of helium was flowing, both frequencies should have increased and the frequency shifts should have been related in an easily calculable way. Reppy found that the frequency shifts did not obey that relation, as he reported in May in *Physical Review Letters*.

Now, in what appears to be the coup de grâce for supersolidity, Chan has refined his first experiment with porous glass and found that nothing flows. Not only that, but he and postdoc Duk Kim have explained how stiffening could have produced the signal reported in the 2004 *Nature* paper after all.

In the 2004 experiment, a gap remained between the porous glass and a thin plate that formed the bottom of the can, they say. That gap made the sample cell flexible and left room in which helium could accumulate. And when solid helium in that gap stiffened, it would glue the glass and the plate together more tightly, amplifying the stiffening effect. Simulations show that a mere 20% increase in the stiffness of that helium layer would account for the entire frequency shift seen in the 2004 experiment.

To avoid that effect in the new experiment, Chan and Duk Kim did not place the Vycor in a can. Instead, they coated the glass with epoxy resin, producing a stiff sample cell with no large empty spaces. Once filled with solid helium, that cell should have been much more sensitive to flow than to stiffening. But when the researchers set it twisting and cooled it, they saw no frequency jump or sign of flow, as they report in a paper in press at *Physical Review Letters*.

Such gluing effects could explain the earlier results on oscillators filled with bulk helium, Chan says. So his team fashioned much stiffer sample cells from stainless steel tubing. This time, they saw tiny frequency shifts that were only marginally bigger than would be produced by a 20% increase in the helium's stiffness, as they reported in January in *Physical Review B*.

All told, the data now suggest that stiffen-

ing can explain away nearly all the signs of supersolidity, Chan and others say. “I think that the vast majority of these are elastic effects of some sort that we’ve been too stupid to realize,” Reppy says.

One experiment cannot easily be discounted, physicists say. In 2010, Eunseong Kim and colleagues placed a torsional oscillator in a refrigerator that could spin like a top. They found that the overall rotation suppressed the presumed signals of supersolidity (*Science*, 19 November 2010, p. 1033). That’s what should happen if the flow is real, because

rotation should squelch quantum flow, as they reported in *Science*. Chan and others say that experiment should be repeated with a double oscillator to see if the signals have anything to do with flow.

When discussing the new results, Chan sounds regretful about the hoopla and debate that his research sparked. “I’ve been leading people through the desert for 10 years,” he says with a nervous chuckle. “With a name like Moses, they should have known better.”

But others praise Chan for his honesty and openness. “It’s very rare for the work

that refutes the original claim to come from the same scientist that made the claim,” Illinois’s Leggett says. “I think he’s behaved in an exemplary manner. It’s almost a textbook example of scientific integrity.”

All the effort spent on supersolidity has not been for naught, ENS’s Balibar says. Solid helium’s softness at temperatures above 0.2 kelvin—or “giant plasticity”—is itself a surprise that likely has quantum-mechanical roots, Balibar says. But, he acknowledges, giant plasticity won’t be as exciting as a crystal that flows would have been. —ADRIAN CHO

JAPAN

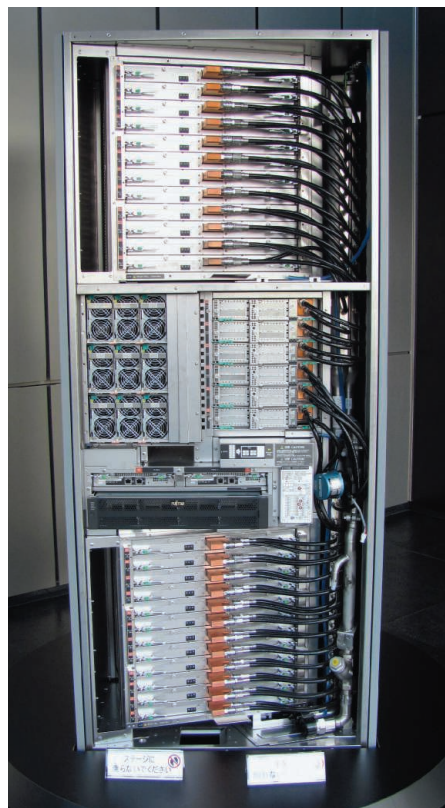
Utility Sacrificed for Speed, Supercomputer Critics Say

KOBE, JAPAN—Japan’s K computer made headlines in June 2011 as the world’s fastest supercomputer and again last November when it became the first computer to top 10 petaflops—or 10 quadrillion calculations per second—solving a benchmark mathematical problem. But by the time it opened for general use last week, it had lost those bragging rights. In June, the IBM Sequoia supercomputer built for the National Nuclear Security Administration at Lawrence Livermore National Laboratory in Livermore, California, became number one, notching a top speed of 16.32 petaflops. And now, after a year of testing and software development, as the \$1.4 billion K computer is put to work on real-world problems, some scientific users say it was too narrowly built for speed.

Being number one in the leapfrog supercomputer race “is as transient as a burst of fireworks,” says Takafumi Matsui, a planetary scientist at Chiba Institute of Technology in Narashino, near Tokyo. He thinks the priority should have been on meeting the needs of scientific users. “There is no connection between being number one in terms of computing speed and producing scientific results,” he says.

In planning since 2006, the K computer project aimed to advance the nation’s computer capabilities and provide a tool for the scientific and business communities, says Ryoji Noyori, a Nobel Prize-winning chemist who heads RIKEN, Japan’s network of national laboratories. The K computer is located at the RIKEN Advanced Institute for Computational Science here.

But Jun Makino, a theoretical astrophysicist at the Tokyo Institute of Technology, says that planners set hardware benchmarks without considering how researchers would use the computer. “Basically, they didn’t explore dif-



Built for speed. Japan’s K computer comprises 864 of these water-cooled cabinets.

ferent ways to design the machine for different applications,” Makino says. Had knowledgeable users been involved, the design of the processors and other key hardware components could have been tweaked to improve the performance for actual applications, instead of being built for pure speed on a benchmark problem. He believes key users and hardware designers should have jointly decided on basic design concepts to “try to find the best combination of software and hardware.”

Matsui is even more critical, saying the project was primarily to benefit Japan’s computer manufacturers. (Fujitsu built K and provided the key semiconductors; NEC was involved in planning the computer but later dropped out.) Developing supercomputer technology “was the biggest reason” for the project, he says.

Many, if not most, users are just happy to have a faster computer. Shigeyuki Yokoyama, a biophysicist who heads the RIKEN Systems and Structural Biology Center in Yokohama, says he wants greater computing power but does not have the expertise to contribute ideas for a supercomputer’s architecture. He is planning on using the K computer to study drug-protein binding, and for his purposes, “faster is better.” And he thinks a top-down approach was needed: Forming a consensus among the high-level users would have taken time, “leaving end users like me waiting for many years,” he says.

Despite any shortcomings, there is no shortage of applicants to use the K computer. Masahiro Seki, director of the Research Organization for Information Science & Technology in Tokyo, which will evaluate proposals, says that there were 227 applications for the first 62 slots. Makino is among those lining up. It is 500 times faster than the computer his group is using for simulations of the formation and evolution of dark matter halos, structures that envelop galaxies. “It’s the fastest computer available in Japan,” he says, and it will allow them to scale up simulations.

Planning for the next next-generation supercomputer has gotten off to a better start, Makino says. He and other users are represented on the three key working groups. He hopes user needs will be better reflected in the eventual design. —DENNIS NORMILE



CLIMATE CHANGE

Researchers Struggle to Assess Responses to Ocean Acidification

MONTEREY, CALIFORNIA—Unlike many areas of global change, there's no argument that rising CO₂ emissions will make the world's oceans more acidic. Average pH in surface waters is now 8.1—a 30% increase in acidity since the start of the industrial revolution—and forecasters say it could drop to 7.8 by 2100 if carbon emissions continue unabated. But how fisheries and other marine life will respond is far from clear.

Building a better crystal ball to gauge such effects emerged as a central challenge facing scientists attending the Third International Symposium on the Ocean in a High-CO₂ World here last week. “Looking realistically into the future is hard,” says Jean-Pierre Gattuso, a biogeochemist at the French National Center for Scientific Research in Villefranche-sur-mer. “But we have to try if we want to understand what acidification will mean.”

The meeting was a coming-out party of sorts for scientists interested in the biological implications of the chemical changes occurring as the oceans absorb huge and growing amounts of atmospheric carbon dioxide. Just 8 years ago, an inaugural symposium on the topic in Paris drew only 125 researchers from 20 nations; this year, more than 550 scientists from 40 nations showed up. The field is getting “much bigger and more competitive,” Gattuso says.

Acidification researchers are also shifting their focus. To date, many experiments have involved simply plopping sea creatures into laboratory tanks full of acidified water for a few days or months to see how they respond. Many species suffer, researchers reported. Fish and shellfish larvae exposed to

more acidic waters, for example, often fail to thrive: They don't grow as big or live as long as those born in more alkaline waters. But some species show substantial resilience, reported biologist Sam Dupont of the University of Gothenburg, Kristineberg, in Sweden. After he used acidic water to completely dissolve the shells of developing sea urchins, for instance, the urchins were able to regrow them and live normally once they were returned to normal seawater.

Such limited studies, however, “can't really tell you whether a species has the capacity to adapt to acidification, or how pH changes affect a larger ecosystem,” says marine scientist Gretchen Hofmann of the University of California, Santa Barbara.

One approach to leaping those limitations is to go back to the future, by looking for times in the fossil record when ocean ecosystems experienced similarly dramatic carbon dioxide-driven changes. One popular candidate, known as the Paleocene-Eocene Thermal Maximum (PETM), occurred 55 million years ago during rapid global warming (*Science*, 18 June 2010, p. 1500). Increasingly corrosive bottom waters appear to have helped drive many bottom-dwelling species extinct during the PETM, reported

Bubble bath. Carbon dioxide seeps off Italy give scientists a peek at what a more acidic ocean could mean for marine life.

paleontologist Paul Bown of University College London. But what happened at the ocean's surface is less clear. The fossil record suggests that many species of phytoplankton—the tiny plants at the base of the marine food chain—also disappeared but were replaced by other species, with little change in overall diversity.

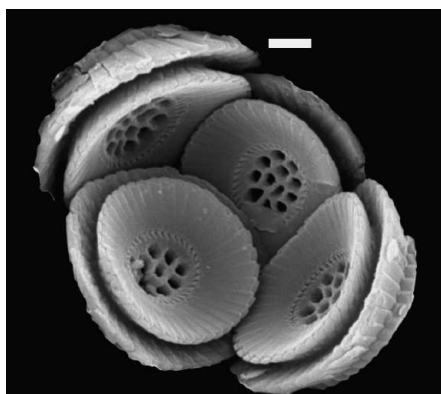
But such coarse measures can't tell you how ancient acidification might have affected reproduction or growth patterns in these marine communities, Bown says. To get that more detailed view, Bown and his colleagues have been analyzing some exquisitely preserved fossils of PETM phytoplankton called coccolithophores, which surround themselves with shieldlike plates of shell. By studying some closely related living species, the researchers found that they could estimate ancient coccolith growth and reproduction patterns by painstakingly counting the plates on individual fossils. (The number increases as the organisms grow.) So far, preliminary studies haven't found deformed shells or other dramatic signs of lower pH, but Bown cautions against taking that as a sign that modern acidification won't be a problem. Change in the PETM moved “much, much slower than today,” he says.

To evaluate acidification's potential impact on modern ecosystems, researchers are also seeking out rare places where the future has already arrived, such as natural sea-floor seeps where bubbling carbon

dioxide gas dramatically lowers the pH of surrounding seawater. Studies of seeps off Papua New Guinea have already shown that tropical coral reefs in acidified waters have fewer species and slower growth rates than nearby reefs (*Science*, 13 July, p. 146).

Similar changes are apparent on rocky bottoms at seeps in the more temperate

Mediterranean waters, reported marine biologist Kristy Kroeker of Stanford University in Palo Alto, California. Off the coast of Italy, she's been studying shallow-water seeps nestled beneath a towering Medieval castle. There, small, fast-growing invertebrates and



Time machine. Coccolithophore fossils might help forecast how future pH changes will affect ocean phytoplankton.

weedy filamentous algae are displacing an array of larger, flashier shell-building species. That could be bad news for tourism if acidifying waters spark similar changes elsewhere, she says: “People are going to be much less likely to pay to go see a bunch of fleshy seaweeds.”

A third emerging approach is to study the evolutionary implications of acidification by pursuing longer and larger experiments. Several teams, for instance, have launched years-long “rapid evolution” experiments to evaluate how organisms adapt to acidified conditions. In one, a team led by David Hutchins of the University of Southern California in Los Angeles spent nearly 4 years raising about 600 generations of an important marine nitrogen-fixing bacterium, *Trichodesmium*, in either current or more

acidic conditions. Bacteria in the acidified tanks greatly stepped up their nitrogen-fixing activity over time, he reported. And, surprisingly, they didn’t revert back to lower activity when moved back into the less acidic tanks, “suggesting true adaptive changes,” he says. Genetic studies backed up that idea, he reported: Acidification could have “major implications for the future of the marine nitrogen cycle” by altering bacterial populations.

Other researchers are taking to the field to find wild populations that have already adapted to acidic waters, which occur naturally in some parts of the ocean. “Basically, space is substituted for time, and we just go see if Mother Nature has already created populations that are fine-tuned,” says Hofmann, who is helping organize one such

collaboration, the Ocean Margin Ecosystems Group for Acidification Studies (OMEGAS). It is studying sea urchins and mussels along North America’s West Coast, where waters tend to get more acidic along a south-to-north gradient from California to Canada. As a result of natural upwelling, the waters off Oregon “are like it is already 2100,” says Hofmann. She reported preliminary studies showing that sea urchin larvae there tolerate acidic water better than their California cousins. That early picture from OMEGAS’s crystal ball “is good news,” Hofmann says, because it suggests that the thorny urchins have “the resilience and genetic variation sufficient to tolerate” global acidification. Still, she says, that’s an experiment she’d rather not run.

—DAVID MALAKOFF

CHEMISTRY

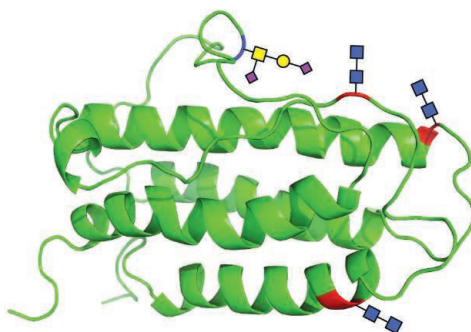
‘Awesome’ Synthesis Could Boost Protein-Based Drugs

Like mountain climbers always on the lookout for a higher, more dramatic ascent, synthetic chemists constantly strive to make larger, more complex molecules. The challenge forces them to invent novel chemical techniques, and the molecules—once in hand—can spur further studies that lead to broad insights in biology and medicine. “When you climb a mountain, you don’t know what’s on the other side. But at least you can find out,” says Samuel Danishefsky, a synthetic chemist at the Memorial Sloan-Kettering Cancer Center (MSKCC) in New York City and Columbia University.

Now Danishefsky and colleagues have scaled a peak that offers a good view of not only their latest molecular target—a protein known as erythropoietin (EPO)—but a host of other molecular landmarks as well. Last week, the MSKCC-Columbia team reported online in *Angewandte Chemie* that for the first time they had synthesized EPO with a uniform coating of sugar chains that decorate the outside of the natural molecule. EPO is a hormone produced by the kidneys that stimulates the production of red blood cells. It’s also administered as a drug, sometimes called a biologic, to anemia patients, as well as those with cancer who have undergone radiation and chemotherapy treatments that can damage red blood cells.

“EPO is the most complex biologic synthesized to date,” says Laura Kiessling, a chemist at the University of Wisconsin, Madison, who calls the accomplishment “remarkable.” Peng George Wang, a syn-

thetic chemist at Georgia State University in Atlanta, agrees that the new synthetic ascent is “an awesome achievement,” because the researchers not only synthesized a complex protein but also decorated it with a uniform set of sugars—a feat that has long been out of reach. A decade ago, “we could not imagine it could be a target. It was just too complicated,” Wang says. Danishefsky notes that the feat rests on a decade of advances in fab-



New feat. Synthesis of proteins with uniform sugar chains (colored shapes) should aid drug development.

ricating portions of the protein, linking them together, and then coming up with novel chemical techniques to tie sugar chains on at precise locations. Although the version of EPO the group made has shorter sugar chains than those typically found in organisms, biochemical studies showed that it carried out the same function of stimulating the production of red blood cells.

But Peng and others argue that the syn-

thesis of EPO has a broader significance. Medicinal chemists have long synthesized small druglike molecules and then tweaked their structures and watched to see whether these changes reduced the compound’s toxicity, improved the amount of time it circulated in the body, or changed other hallmarks of a good drug. But this tweak-and-peek strategy has largely been impossible with complex biologics. Take EPO, for example. When harvested from cell cultures for use as a drug, it forms a mixture of molecules in which three of the four sugar chains differ widely.

Researchers have long known that EPO’s sugars are essential to its function. But it has been difficult to tease out the precise role of all the different sugars in the various chains. Now, because the new synthetic techniques make it possible to create single “glycoforms” of the protein, researchers can synthesize different versions and systematically test them to see how the protein’s function changes. “That opens up an enormous opportunity to take the chemistry and use it to probe a biologic’s secrets,” Danishefsky says. His group is now doing just that, he says.

Wang predicts that chemists will soon extend the new synthetic sophistication far beyond EPO, with repercussions including illuminating how glycoproteins function and designing novel medicines. “I think the implications for biomedical research are enormous,” he says.

—ROBERT F. SERVICE

BIOMEDICINE

Pharma Firms Push for Sharing Of Cancer Trial Data

Of the hundreds of compounds that undergo clinical trials for use in cancer therapy, less than 5% make it to the pharmacist's shelf, a success rate lower than that for experimental drugs in most other fields of medicine. Now, a consortium of pharmaceutical companies hopes to improve that frustratingly inefficient enterprise by getting cancer researchers to share data from clinical trials. Called DataSphere, the effort aims to create a repository of data sets from cancer trials conducted by drug companies, academic labs, and other organizations. The initiative, being organized by the CEO Roundtable on Cancer—a nonprofit convened in 2001 by George H. W. Bush—has been kick-started with two data sets contributed by pharma giant Sanofi. Other companies, as well as universities, are expected to contribute data in the coming months. The architects of the effort hope to make the repository available to outside researchers by April of next year.

"It's taking \$2 billion or more to bring a drug to market. At the same time, we've got about the same number of people dying from cancer that we did 30 to 40 years ago. We have got to get more innovative in how we

away their competitive advantage in the market. And academic researchers are reluctant to make their data sets freely available because it may hamper their ability to get as many publications out of the data as possible. Then there are concerns about violating patient confidentiality.

But as drug development costs continue to rise, the industry appears to be more ready to cooperate. Just last month, a group of 10 pharma companies announced that they would work together through a newly formed nonprofit named TransCelerate BioPharma to standardize clinical trial formats and execution in many ways, from patient recruitment to how to record data. The hope is that it will be easier to compare data across drug trials if the data are in a standard format.

Many of the same companies are behind DataSphere. The consortium has spent the past several months discussing how to imple-

generic compound—to which the experimental drug is being compared. Pooling these data sets and analyzing them could provide valuable insights, says Mace Rothenberg, senior vice president for clinical development and medical affairs at Pfizer Oncology and a member of the consortium.

For instance, variations in outcomes for the same comparator drug in different trials "may have been due to differences in the

patients," Rothenberg says. "As you get larger and larger sample sizes, you can begin to say that there's something emerging here. You can start identifying subgroups of patients that respond in specific ways to a drug."

Hugh-Jones says DataSphere could eventually help researchers devise ways to use the comparator arms of past trials as proxies in new trials, bringing down the number of patients that companies would need to recruit to test a

particular drug. This would require approval from the U.S. Food and Drug Administration (FDA), he notes.

To address privacy concerns, Sanofi will strip identifiers such as a person's name, address, and date of birth from the two data sets it will initially contribute, Hugh-Jones says. One is the comparator arm of the phase III trial for cabazitaxel, which was approved by FDA in 2010 for use in treating metastatic hormone-refractory prostate cancer. The other is the comparator arm for the phase III trial of iniparib, an unsuccessful candidate for treatment of certain types of breast cancer.

Hugh-Jones says he has been reaching out to patient advocacy groups about DataSphere in the hope that they will help popularize the sharing of clinical trial data. Many patients are appalled to learn that the data are "collecting dust in our warehouses," Hugh-Jones says. "At the same time, you have to assure patients that the data will be held in a secure environment and will be shared responsibly."

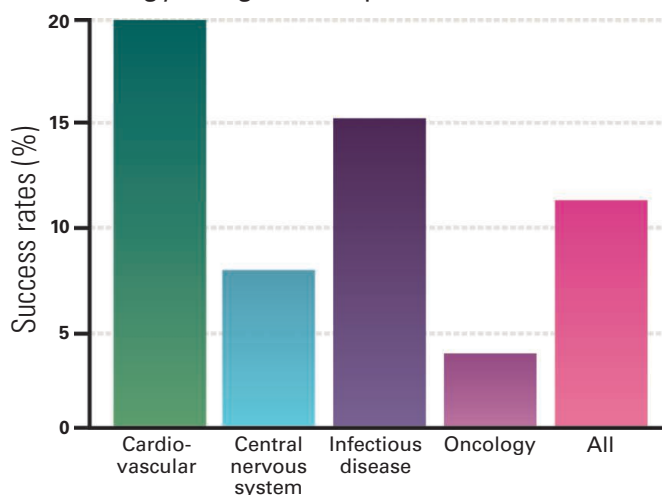
DataSphere could benefit academic scientists immensely, says Amy Abernethy, an oncologist at Duke University School of Medicine in Durham, North Carolina. "It will help with hypothesis generation and stimulate new directions for inquiry," she says. "At Duke, we are certainly advocating to contribute. My colleagues have been positive, even when I suggest that it is 'their trial' that would be donated to DataSphere."

—YUDHIJIT BHATTACHARJEE



Together. Hugh-Jones says sharing data will help accelerate drug development.

Oncology Drug Development Is Inefficient



Hit or miss. Cancer drug candidates have an abysmally low success rate.

develop cancer drugs," says Charles Hugh-Jones, the North American vice president of medical affairs at Sanofi's oncology division.

Researchers have talked about sharing clinical trial data for some time, yet pharma companies have worried that they could give

that pooling data could cut the current costs of developing a drug by 10%.

Initially, drugmakers and academic institutions are expected to contribute data from the comparator arms of clinical trials; that is, data relating to the conventional therapy—often a

Mysteries of the Brain

NEXT WEEK, TENS OF THOUSANDS OF researchers will make their way to New Orleans, Louisiana, for the annual meeting of the Society for Neuroscience, a testament to both the vitality of this discipline and how little we know about the body's most complex organ. To identify and explore some of the brain's enduring mysteries, Senior Editor Peter Stern and the news staff at *Science* have consulted with neuroscientists from our Board of Reviewing Editors and elsewhere. The brain mysteries we chose for a closer examination here encompass medical science, evolutionary biology, cognitive science, and more—and leave many provocative questions to another time (see p. 39).

—LESLIE ROBERTS AND JOHN TRAVIS

How Are Memories Retrieved?

So much of memory is a puzzle. How can the experiences of a lifetime—the sights and sounds, people and places, successes and failures—be recorded in the soft tissue of the brain? How can those memories persist for decades even as the neurons that encode them undergo constant molecular remodeling? And how can we (more often than not) recall a particular bit of information almost instantaneously, and with little prompting?

This last question may be the most mysterious of all. “Retrieval is such a rich phenomenon,” says Michael Hasselmo, a neuroscientist at Boston University. “You get a reminder from somebody that’s maybe just a word and you somehow turn it into a rich internal movie of events that you’re moving through with a perspective and a location and a sense of time passing.” Our memories are part of what makes each of us unique (see p. 35), and they give us a sense of self-identity and continuity as we move through life. “Without our memories, we’re just zombies,” says György Buzsáki, a neuroscientist at New York University in New York City.

The neuroscience of memory is a complex and contentious area, but most researchers agree on a broad-brush account that goes something like this, at least for episodic memories, or memories of events. These memories are initially encoded and stored mostly in the hippocampus, deep inside the temporal lobe of the brain. For long-term storage, memories are filed away to other areas, including the neocortex, the thin sheet of tissue on the surface of the brain. A memory of any given event, the thinking goes, is represented by a sparse and scattered network of neurons, such that the sights, sounds, and emotions associated with the experience may each reside in a different location. To recall that memory, the brain must somehow reactivate just the right subset of neurons. Many details of this process are not known (or are disputed). Even so, some researchers say it’s time to revise some aspects of the standard view—such as the notion that the hippocampus is not involved in retrieving older episodic memories, and that memories become fixed and unchangeable once transferred to the neocortex. Newer work suggests a far more fluid role of memory, and one in which retrieval plays a crucial role in shaping memory over time.

So what should researchers look for if they hope to learn how the brain recalls the past? One clue comes from functional magnetic resonance imaging (fMRI) studies of the human brain suggesting that remembering reactivates some of the same neural circuitry as the original experience. Recalling a face, for example, activates a part of the fusiform gyrus thought to specialize in face recognition. Recalling a place evokes a different pattern of brain activity that includes the parahippocampal gyrus, an area that lights up when people view images of landscapes and other scenes.

“We have a pretty good idea that the brain uses the same machinery for remembering that it does for experiencing things,” says Loren Frank, a neuroscientist at the University of California, San Francisco. When it comes to episodic memories, Frank says, what’s stored in the brain are little snippets of the experience that can be compiled into a kind of highlight reel. The neural signature of memory retrieval, Frank argues, should look much like the neural signature of the actual experience played in fast-forward.

There’s disagreement about how fast the replay should be, but several labs, including Frank’s, have found something like this in the

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Downloaded from www.sciencemag.org on October 5, 2012

brains of rats. One example is a 2008 study from Buzsáki and colleagues, in which the researchers trained rats to alternate taking left and right turns at a particular point in a maze. They found, as others had before, that each path evoked a specific sequence of firing by so-called place cells in the rodents' hippocampus. In a twist, the researchers then gave the rats a break between turns in the maze. They found that during this time, their place cells fired in sequences that predicted which direction they would turn on their next run in the maze. One sequence played when the rat had a left turn coming up, and a different sequence played when a right turn was next (*Science*, 5 September 2008, p. 1322).

Do these hippocampal firing sequences represent the rat reminding itself what it needs to do next? Buzsáki thinks so. "Plans are based on memories," he says. Buzsáki speculates that such sequences also play a broader role in recalling episodic memories. "The hippocampus is like a librarian," he says: Its job is to record new experiences, help file them away to the neocortex, and later retrieve them on demand. In the case of episodic memories, the firing sequence of hippocampal cells might serve the same purpose as the identifying bar code on the spine of a book, indicating which subset of neocortical neurons represents a given memory.

Recent studies support the concept that memory retrieval involves reactivating small but specific sets of neurons. In one, published online on 22 March in *Nature*, researchers used genetic engineering meth-

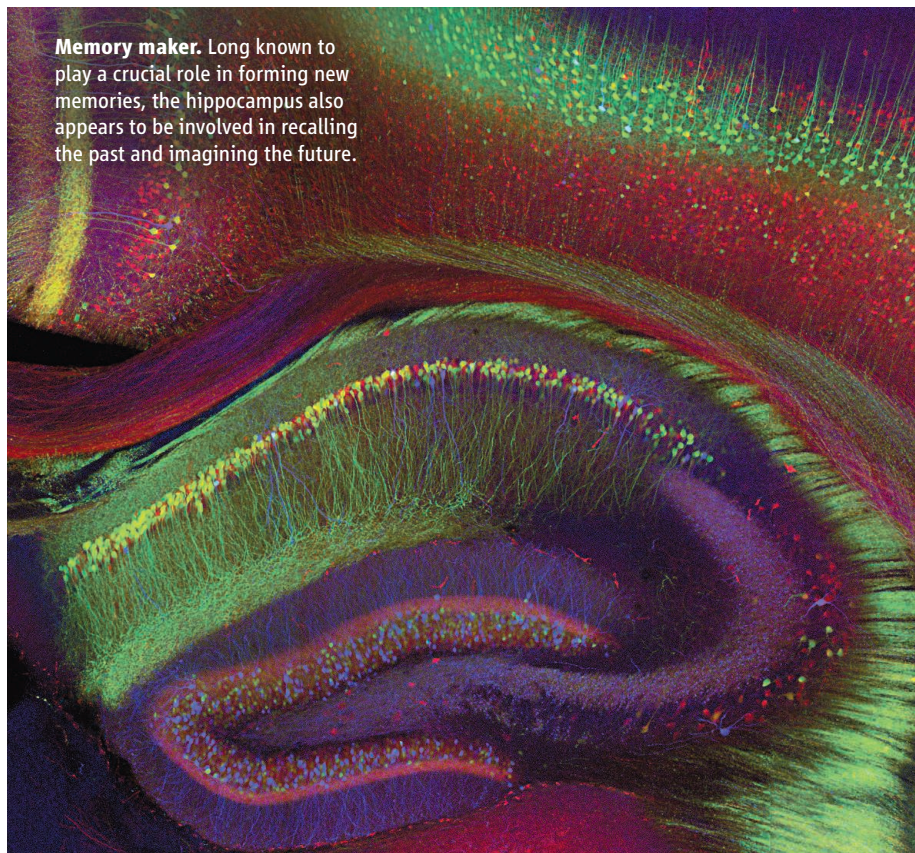
Online

Podcast interview with Greg Miller (http://scim.ag/pod_6103).

ods to tag neurons in the hippocampus that were activated as a mouse learned to associate a flash of light with an impending shock. When the researchers then reactivated those same neurons with a pulse of laser light delivered by an optical fiber, the mice froze in fearful anticipation even though they hadn't seen a flash of light.

Bridging the gap between such rodent studies and the human brain isn't easy. In rare cases, neuroscientists have taken advantage of monitoring electrodes placed in or on the brains of epilepsy patients awaiting surgery. Such studies are done only when they don't interfere with medical care. In a 2008 study, researchers asked patients to watch several short video clips from TV shows and movies and then recall as many of them as possible a short time later. Individual neurons in the hippocampus seemed to develop a preference for a specific clip, firing strongly a second or so

Memory maker. Long known to play a crucial role in forming new memories, the hippocampus also appears to be involved in recalling the past and imagining the future.



before a patient named a clip, say, from *The Simpsons*, but not when he or she recalled any of the other clips (*Science*, 3 October 2008, p. 96). The findings provide some of the best evidence that reactivation of specific hippocampal neurons is involved in the conscious experience of memory retrieval.

Memory researchers have also begun to use methods borrowed from machine learning to analyze patterns of brain activity from fMRI scans on a finer scale than conventional methods allow (*Science*, 13 June 2008, p. 1412). "We can actually now identify individual memory traces by the pattern of activity in areas of the brain such as the hippocampus," says Eleanor Maguire, a cognitive neuroscientist at University College London. "We can predict in an experiment the memory that someone is recalling." (But only when the choices are limited: The technology is nowhere close to being able to read out any fleeting memory that crosses someone's mind.)

Maguire says that work by her group and others is beginning to challenge the dogma that the hippocampus is not involved in recalling older episodic memories. The overarching role of the hippocampus, she hypothesizes, is to pull together the various aspects of a memory residing in different regions of neocortex and bind them into a coherent scene in the

mind's eye. Recent studies suggest that the hippocampus even does this when people ponder imaginary scenarios. By stitching together pieces of the past, Maguire says, the hippocampus enables us not only to vividly remember but also to envision possible futures.

Another recent challenge to traditional views of memory retrieval comes from research suggesting that memories can be incrementally strengthened, weakened, or otherwise altered each time they're recalled. "That work has changed the way people think about the persistence of memory," says Yadin Dudai of the Weizmann Institute of Science in Rehovot, Israel. Such findings point to a more malleable memory system in which retrieval presents an opportunity to update old memories in light of new experience. Our repository of memories may be less like a library and more like Wikipedia, where each entry is open to editing anytime it's pulled up.

This type of plasticity may be crucial for fitting new memories into the existing network of old memories, says Howard Eichenbaum, a neuroscientist at Boston University. "Everything you learn has to fit in with what you already know," Eichenbaum says. How the brain accomplishes that never-ending task is a puzzle scientists have only begun to explore.

—GREG MILLER

Why Is Mental Illness So Hard to Treat?

“The last quarter century has seen many forward strides in the management of patients with mental disease. During this period modes of therapy have been available that are far superior to the old-time method of whirling patients on wheels or ducking them into cold water.” So begins a 1954 article in the *Journal of the American Medical Association* on the calming effects of reserpine, a compound derived from an Indian plant, on psychotic patients in a California mental hospital. Its effects were so remarkable, the authors wrote, that if their findings held up, “reserpine will be the most important therapeutic development in the history of psychiatry.”

Serious side effects ultimately prevented reserpine from being the game changer its early champions had envisioned, but other drugs discovered around the same time—including lithium for bipolar disorder, monoamine oxidase inhibitors for depression, and chlorpromazine for schizophrenia—gave clinicians their first real weapons against mental illness.

Flash-forward to the 21st century. Current psychiatric drugs are not much more effective than those initial medicines. Pharmaceutical companies have few promising candidates in the pipeline and show signs of giving up (*Science*, 30 July 2010, p. 502). Meanwhile, mental illness remains

a major cause of disability throughout the world. Why has it been so hard to develop new treatments?

“We just don’t know enough,” says Thomas Insel, director of the U.S. National Institute of Mental Health (NIMH) in Bethesda, Maryland. “Research and development in this area has been almost entirely dependent on the serendipitous discoveries of medications. From the get-go, none of it was ever based on an understanding of the pathophysiology of any of the illnesses involved.”

It’s little wonder that uncovering the roots of psychiatric illness has not been easy. Not only is the brain the most complex organ in the body, but it’s also harder to study. Doctors and researchers can’t biopsy a patient’s brain as they would a diseased kidney or swollen prostate. Genetic studies of psychiatric disorders may yet uncover solid leads for drug developers, but so far they’ve mostly uncovered bewildering complexity.

There’s also the question of how well disorders of the human mind can be studied in other species. Many of the animal models of mental illnesses in use today were developed decades ago to screen for compounds with effects similar to those of the early antidepressant and antipsychotic drugs, says Steven Hyman, who directs the Stanley

Center for Psychiatric Research at the Broad Institute in Cambridge, Massachusetts, and preceded Insel as NIMH director. In the “forced swim test,” for example, researchers put a rat or mouse in a tub of water and clock how long it takes for the animal to stop struggling and just float, as if it’s given up. “This is called behavioral despair,” Hyman says. Of course, nobody knows if the rat experiences anything like what a person in the grips of depression experiences, but rats given imipramine, one of the early antidepressants, struggle longer. The forced swim test has in fact identified other drugs that turn out to have antidepressant effects in humans, Hyman says, but by relying on a test designed to find imipramine-like effects, researchers may have missed drugs that work by means of other, potentially more effective mechanisms.

Hyman, Insel, and others argue that too much effort has been spent searching in the narrow beam of light cast by the early psychiatric drugs, looking for similar compounds or tweaking their chemistry to eke out improvements in efficacy, reduce their side effects, and—not insignificantly—preserve the revenue stream generated by patent-protected drugs. The field of psychiatric medicine, it seems, got lucky early on, and then it got in a rut.

However, several new tools and new ways of thinking could help the field gain new traction. One example, Insel says, comes from drugs that target receptors for the neurotransmitter glutamate, which recent evidence suggests can reduce hopelessness and suicidal ideation in people with depression far faster than current drugs do. “That story, while it’s still developing, is extraordinary because it tells us we need to rethink our expectations,” Insel says. “It may be possible to treat this in hours instead of weeks.”

Another encouraging inroad into depression comes from deep brain stimulation (DBS), in which surgeons implant electrodes in brain regions thought to be involved in regulating emotion and cognition. The approach is still experimental, and only severely depressed patients who’ve failed to respond to less invasive treatments are eligible, but DBS seems to help about three-quarters of them, says Helen Mayberg, a neurologist at Emory University in Atlanta and a pioneer of this therapy. The success of DBS, Mayberg and others suggest, undermines the decades-old concept of mental illness as primarily a chemical



Don't despair. Progress on new treatments for disorders of the mind has been frustratingly slow, but some researchers see new hope on the horizon.

imbalance—too much or too little serotonin floating around the brain, for example—and points instead to faulty neural circuits as the core problem. In their quest for the next generation of treatments, researchers should focus less on single molecules and individual brain regions, Mayberg says, and think of the brain as “a dynamic system that has to be properly choreographed.”

One powerful new tool for examining neural circuits at the cellular level is optogenetics, which combines laser optics and genetic engineering to stimulate or inhibit specific classes of neurons in rodents and monkeys. Researchers have employed optogenetic methods in mice to investigate the mechanisms of DBS therapy for Parkinson's disease (*Science*, 20 March 2009, p. 1554) and to study the neural circuits involved in addiction, anxiety, and other conditions.

On a larger scale, human brain imaging research is illustrating that psychiatric conditions are multifaceted, with different symptoms that can be traced to different networks of brain regions, says Cameron Carter, a cognitive neuroscientist at the University of California (UC), Davis. The delusions and hallucinations of schizophrenia, for

example, may involve malfunctions in one network, while disordered thinking and other cognitive problems involve another. This brain-based view is at odds with the traditional approach of diagnosing disorders according to the behavioral problems and inner anguish they cause—the approach taken by psychiatry's go-to diagnostic guide, the *Diagnostic and Statistical Manual of Mental Disorders (DSM)*, published by the American Psychiatric Association. “There's no doubt that these categories [in the *DSM*] don't map very well onto nature,” Carter says. An effort to create a new diagnostic scheme rooted in biology, spearheaded by NIMH, is already under way (*Science*, 19 March 2010, p. 1437).

And studies of the human genome may yet lead to innovative drugs for mental illness. Researchers have identified hundreds of genetic variants that increase the risk of autism, for example. Making sense of this deluge of new data is a challenge, but there

“Research and development in this area has been almost entirely dependent on the serendipitous discoveries of medications.”

—THOMAS INSEL,
U.S. NATIONAL INSTITUTE
OF MENTAL HEALTH

are some hints of convergence: Many of the autism risk genes appear to be involved in common biological functions, such as synaptic signaling and brain development. Researchers now have novel tools at their disposal to follow up on these leads, including the ability to create neurons from reprogrammed stem cells from patients

(*Science*, 26 November 2010, p. 1172). Gene expression profiling—investigating the activity of thousands of genes—in these human neurons could help researchers identify the biological pathways disrupted by autism risk genes and screen drugs to correct them, says Daniel Geschwind, a neurogeneticist at UC Los Angeles. “I'm extremely optimistic that [by] using a combination of these methods we're going to be developing new classes of drugs,” he says. “I think we're on the threshold of something really exciting.”

—GREG MILLER

Why Are Our Brains So Big?

With an average volume of about 1400 cubic centimeters, the human brain is more than three times as large as that of the chimpanzee, our closest living evolutionary cousin. And although the brains of whales and elephants are bigger in absolute terms, once adjusted for body weight the size of the *Homo sapiens* brain outstrips that of any other animal.

For the past 2 decades, the leading explanation for why natural selection bestowed such generous largesse on the human noggin has been the social brain hypothesis. The researcher with whom the idea is most closely associated, psychologist Robin Dunbar of the University of Oxford in the United Kingdom, has argued that brain size—and particularly the size of the brain's neocortex—most closely correlates with the size of a species' social group. Keeping track of who is doing what to whom, Dunbar and other researchers argue, requires considerable processing power, and so bigger groups demand bigger brains.

Numerous studies by Dunbar and others appear to support the link between a species' group size and neocortex size, especially among primates (*Science*, 7 September 2007, p. 1344). And recently, a number of brain-

scanning studies in humans and monkeys have found correlations between the size of social networks and that of specific brain areas linked to sociality (*Science*, 4 November 2011, p. 578). One study of people, for example, found a positive relationship between gray matter density and the number of Facebook friends an individual has.

“The social brain hypothesis is widely accepted and has generated a huge amount of research,” says Robert Seyfarth, a biological anthropologist at the University of Pennsylvania. Yet there are competing explanations for our big brains, and Seyfarth and other researchers are concerned that Dunbar's formulation may be too simplistic to account for the complex course of human brain evolution. The social brain hypothesis “now needs to be balanced against other complementary hypotheses and more fully integrated” with evidence from cognitive neuroscience, says Robert Barton, an evolutionary anthropologist at Durham University in the United Kingdom.

Researchers generally agree that our large brains have something to do with how smart we are and why we were able to take over the planet. But if the evolutionary path to a big

brain were easy, one might think that every animal would have one. Yet brains use a lot of energy, and most species have maintained smaller ones throughout their evolution, apparently avoiding the cost of fueling all that processing power.

One key question is whether a species' group size, which is usually considered a proxy for its social complexity, was the driving factor in the evolution of bigger brains, or if it was the other way around: Natural selection could have favored larger brains for other reasons, such as greater innovation in food-foraging and tool-using skills, which then made larger social groups possible.

Richard Byrne, a cognitive neuroscientist at the University of St Andrews in the United Kingdom, argues that great apes like chimps, gorillas, and humans evolved larger brains “to solve challenging food-acquisition problems better than monkeys,” with whom they competed for resources in the wild. His “Machiavellian intelligence” hypothesis, formulated in the late 1980s with St Andrews colleague Andrew Whiten, focused on the cognitive challenges of balancing competition and cooperation within primate groups, and was a forerunner to the social brain hypothesis.

These challenges, Byrne says, led to brains better equipped to understand cause and



The smart set. Macaques live in complex social groups and have brains larger than expected for their body size.

effect—necessary for the development of tool use, such as fishing termites out of trees with sticks as wild chimpanzees do, and understanding the intentions of other individuals, thus making ever-more-complex social relations possible.

A similar emphasis on food-gathering innovations has been argued by biologists Simon Reader of McGill University in Montreal, Canada, and Kevin Laland of St Andrews. Reader says that although he finds the new brain-scanning studies showing correlations between the sizes of certain brain regions and group size “particularly interesting,” there are likely to be “multiple drivers of cognitive evolution” that “need not be mutually exclusive.”

Dunbar, however, rejects the notion that food-foraging innovations alone selected for big brains, and then the capacity to live in large groups “came for free.” Living in large groups, Dunbar says, is “hugely costly” in time and energy.

Beyond offering alternatives to the social brain hypothesis, Seyfarth and some other researchers have also challenged its assumptions. They argue that group size is too crude a measure of the brain-taxing demands of social relationships. “The best predictor of reproductive success is the nature of the social relationships animals form, which are often with a smaller fraction of the entire group,” Seyfarth says, citing his own studies and those of others, especially with baboons.

Recently, a team led by Carel van Schaik, a primatologist at the University of Zurich in Switzerland, has put forward what it calls the “cultural intelligence hypothesis,” which attempts to incorporate a broader range of factors, including an animal’s behavioral flexibility and social learning—the transmission of skills and information within a species. Van Schaik and his colleagues argue that the social brain hypothesis does a poor job of predicting the brain size of primates such as orangutans and aye-ayes, whose social relations are relatively simple and yet who have larger brains than closely related primates living in much more complex social groups. And the team points out that small-brained hyenas and even some bats live in societies as highly complex as those of many primates.

“The social brain hypothesis seems to be too narrow,” van Schaik says, adding that the cultural intelligence hypothesis incorporates the “ecological skills learned through social learning processes, whereas the social brain hypothesis mainly or exclusively focuses on social skills.”

But Dunbar insists that his version of the social brain hypothesis also incorporates

these additional factors. “I don’t really see the difference” between the two hypotheses, Dunbar says. For him, culture is just another “mechanism that allows the social brain to do its work.” And he rejects the idea of treating ecological and social factors as two separate domains.

“The evolutionary logic is that animals need to increase group size to solve one or

“Primates do a balancing act between maintaining group cohesion and using close allies to buffer themselves against the costs of group living, which is no mean feat cognitively.”

—ROBERT DUNBAR,
UNIVERSITY OF OXFORD

more ecological problems, such as the risk from predators, that are best solved socially,” Dunbar says. But living in large groups has its own costs in time and energy, especially when competition arises, he adds. This leads individuals to also form smaller, more close-knit alliances of the type that Seyfarth and others have observed. “Primates do a balancing act between maintaining group cohesion and using close allies to buffer themselves

against the costs of group living, which is no mean feat cognitively,” Dunbar says.

For now, just how the human brain got so big remains a puzzle. Fortunately, natural selection has already made it big enough that this is one mystery we might someday solve.

—MICHAEL BALTER

Why Are You and Your Brain Unique?

Take a seat in any coffeehouse or pub, and odds are it won't be long before you overhear people talking about why someone behaves the way they do. You might catch two mothers talking about whether a child's short attention span was inherited from his father. Or two friends speculating on whether a mutual acquaintance is so neurotic because of the way she was raised. The differences in our individual talents and tendencies, and their origins, are an endlessly fascinating conversation topic.

They're becoming a hot issue for scientists, too. Now that the nature vs. nurture debate has been declared a draw, researchers have turned their attention to how genetics and life experiences interact to make each person's brain unique. Recent work has provided clues about the neural basis of individual differences in behavior, cognition, and even personality, but there's still much we don't know.

Virtually every brain-based trait that's been examined, including general intelligence, personality traits such as extraversion, and the risk of mental illness, turns out to be influenced by the genes one inherits, says Robert Plomin, a behavioral geneticist at King's College London. "You don't find that any [traits] are 100% heritable," Plomin says. "You do find that some are somewhat more heritable than others."

That leaves a lot of room for our brains to be molded by environmental influences. Neuroscientists have long thought that experiences early in life have an especially powerful influence on brain development, and although that's probably true, recent research suggests that even older brains may be more malleable than once thought (see p. 36). Our memories also make each of us unique, and they pose scientific mysteries of their own (see p. 30).

Genes and life experience certainly interact to generate individuality. One much-discussed and debated example

involves the gene for a protein that shuttles the neurotransmitter serotonin back into neurons after it's been released. People who possess a particular variant of this gene were more likely to experience symptoms of depression and suicidal thinking, and were more likely to receive a formal diagnosis of depression—but only when they also experienced a stressful life event, such as the loss of a job or a loved one (*Science*, 18 July 2003, p. 291).

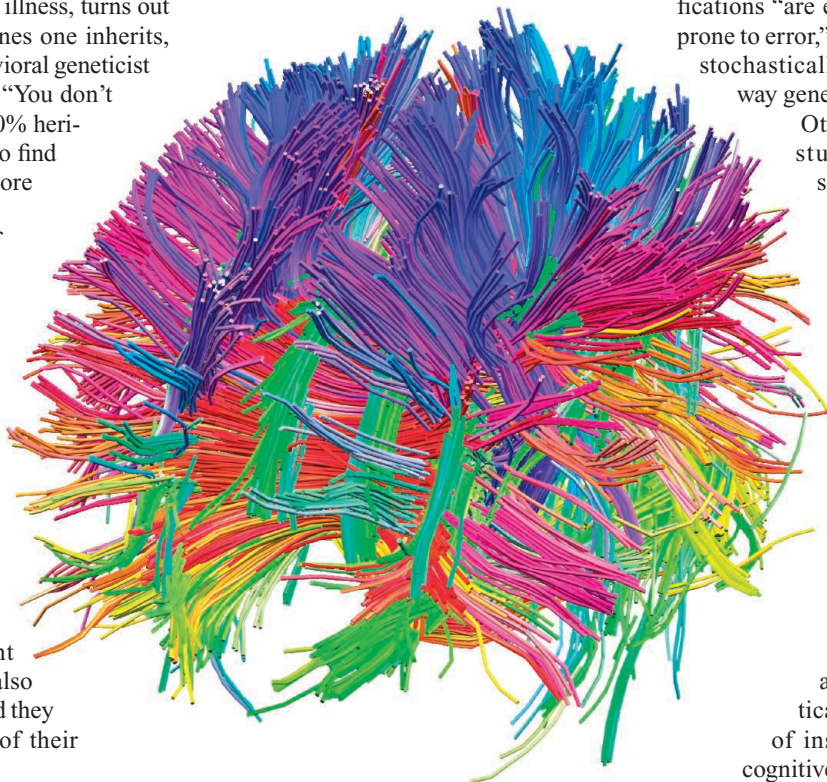
Plomin thinks our genes may also shape our environment. One of the most puzzling findings in behavioral genetics, Plomin says, is the observation that the genetic influence on intelligence appears to be strongest later in life. That is, while studies of children generally find that about 20% of individual differences in intelligence can be attributed to genetics, that figure increases with the age of the group examined and reaches 80% in some studies of older adults. A possible explanation, Plomin suggests, is that genes nudge people toward choices that shape their environment in a particular way, which in

turn affects their intellectual prowess. It may be that what's inheritable about intelligence is not so much raw brainpower, Plomin says, but the propensity to engage in certain activities: "Do you read books and talk to people who make you think more, or do you lobotomize yourself with television?" Such small decisions could snowball over the course of a lifetime. It's a difficult hypothesis to test, but it's not intractable, Plomin says.

Another insight into how genes and the environment can conspire to influence behavior comes from epigenetics, a field concerned with the chemical alterations to DNA and its associated proteins that result in long-lasting changes in gene expression. Recent studies, mostly with mice, have linked physical abuse and other adverse experiences early in life to epigenetic changes that alter the brain's response to future stressful events (*Science*, 2 July 2010, p. 24). Epigenetics may also amplify individuality by interjecting an element of randomness into how genes are expressed that ultimately affects behavior, says Moshe Szyf, an epigeneticist at McGill University in Montreal, Canada. Szyf notes that studies with genetically identical lab mice and with human identical twins have consistently found individual differences in epigenetic alterations to DNA. These epigenetic modifications "are enzymatic processes that are prone to error," Szyf says. "And that can just stochastically create differences in the way genes are expressed in the brain."

Other researchers have been studying another potential source of random variation in gene expression in developing brain cells: so-called jumping genes, wandering bits of DNA that can insert themselves into other genes and alter their function (*Science*, 15 April 2011, p. 300). But so far it's not clear what effect this has on brain function, let alone behavior.

For the most part, neuroscientists have viewed individual differences in brain anatomy and activity as a source of statistical error rather than a source of insight, says Geraint Rees, a cognitive neuroscientist at University College London. Most neuroimaging studies, for example, average brain activity across people, in part because that requires



True colors. New imaging methods that highlight connections between brain regions could yield new clues about what makes each brain unique.

fewer subjects and cuts down on expensive scanner time. But individual variations in the brain aren't hard to find for those who look.

Rees says his interest was piqued by studies finding that the size of the human primary visual cortex can vary up to threefold. He wondered whether that resulted in differences in vision, an idea his lab has been investigating with a combination of optical illusions and functional magnetic resonance imaging (fMRI). At the end of 2010, Rees's group reported online in *Nature Neuroscience* that people with a smaller visual cortex more strongly experience certain illusions in which the apparent size of an object depends on its visual context. The findings suggest to Rees that even something as basic as how we perceive the world around us varies from person to person in subtle ways that can be traced to variations in brain anatomy.

Richard Haier, a neuroscientist at the University of California, Irvine, is one of the few intrepid scientists who've waded into the potentially touchy realm of individual differences in the brain that influence intelligence. His work, beginning in the late 1980s, has identified a network of regions of parietal and frontal cortex whose anatomy and activity correlates with scores on tests of general intelligence. At the same time, Haier's work suggests that this network isn't identical in all individuals with similar intelligence scores. In other words, smart brains may be built in a variety of ways.

The largest study ever undertaken to look at individual wiring variations in the human brain is the Human Connectome Project, a 5-year, \$38.5 million effort funded by the U.S. National Institute of Mental Health. Now in its third year, the project aims to enroll 1200 healthy adults for a battery of behavioral tests and brain scans, including diffusion imaging scans that show connections between regions of the brain. The overall goal is to investigate individual variations in brain structure and activity and how they may correlate with differences in memory, emotion, and other functions, says David Van Essen of Washington University in St. Louis, Missouri, who is one of the project's leaders.

The project will also examine heritability of brain characteristics by enrolling 300 pairs of twins, plus one or more non-twin siblings for each pair. Researchers will collect DNA for genotyping and possibly whole genome sequencing if the cost drops enough by the final year of the project, Van Essen says.

"I'm confident we'll see interesting individual differences."

—DAVID VAN ESSEN,
WASHINGTON UNIVERSITY IN ST. LOUIS

Whether the differences in neural circuitry that make each person unique will be visible at the resolution of MRI scans is an open question. "It's sobering for sure that the resolution is only at the level of a millimeter or two, which means that each voxel contains literally hundreds of thousands of neurons or axons," Van Essen says. (A voxel is the smallest volume of brain tissue discernable in a brain scan.) "But I'm confident we'll see interesting individual differences."

Other researchers are working on far more detailed maps of neural circuitry. Sometimes called microconnectomics, these efforts employ recently developed methods in genetic engineering, automated microscopy, and image analysis to map out the synaptic connections of individual neu-

rons. So far, the approach has been applied only to millimeter-size chunks of tissue in worms and mice, but some researchers see a microconnectome of the human brain as an ultimate if distant goal.

It's not clear what such a circuit diagram would reveal. Proponents think it would explain a great deal about how the brain works and about the nature of individual differences. Critics contend that deciphering brain function from a circuit diagram—no matter how detailed—is like trying to figure out what a computer does by studying its wiring diagram. In both cases, the circuitry may say something about what the machine is capable of, but it's the precise pattern of electricity coursing through it at a given time that determines what it's actually doing.

It seems far off, but there may yet come a day when brain scans and genetic tests can predict—with enough accuracy to matter in the real world—an individual's mental strengths and weaknesses, predisposition to psychiatric problems, or maybe even his favorite color. In the meantime, in the cafes and bars, there will be plenty to discuss.

—GREG MILLER

Can We Make Our Brains More Plastic?

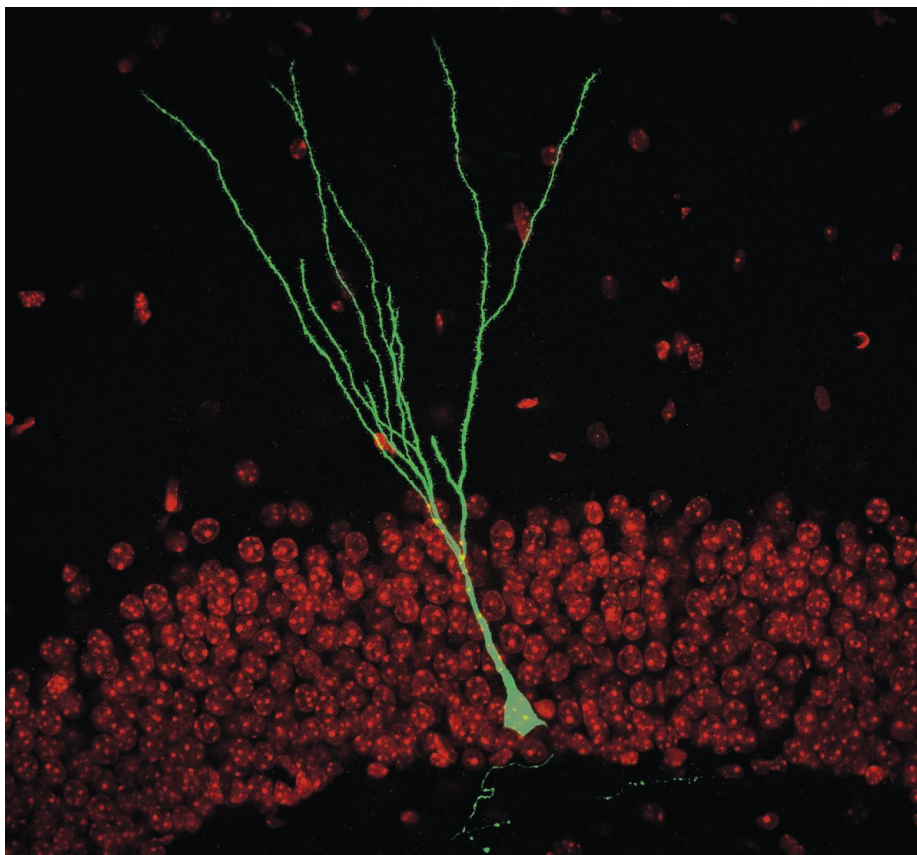
Rewiring the brain is hard work, and as we age it gets even more difficult. A baby exposed to multiple languages can, without apparent effort, become fluently bilingual or even trilingual. Most adults have to work much harder to master new languages, and few are able to achieve the fluency of native speakers.

There are good reasons that our brains become less flexible as they mature: A developing brain gives up some of its plasticity in favor of efficiency and stability. "A fully plastic brain is not very helpful," says Gerd Kempermann, a neuroscientist at the Center for Regenerative Therapies Dresden and the German Center for Neurodegenerative Diseases. "It learns everything but remembers nothing." Too much plasticity may also play a role in some neurological disorders, including epilepsy and schizophrenia.

In certain situations, however, more plasticity could be helpful, making it easier for patients to recover after a stroke or spinal cord injury, for example. And it would be nice to effortlessly pick up the intricacies

of Russian grammar. So, will we one day be able to turn on—and control—our brain plasticity at will?

Neuroscientists have begun to understand a few of the factors that govern the flexibility of certain parts of the maturing brain. By studying the development of sensory systems such as sight and hearing, they have uncovered a network of genes and proteins that influence so-called critical periods, windows of time in which the brain is primed for certain types of input. It is during these critical periods that the brain becomes wired for certain tasks, such as turning the signals received from the eyes into recognizable images, or distinguishing sounds present in spoken language. If a brain doesn't receive the right inputs during a critical period, it is extremely difficult to recover from the deficits that result. Children born with cataracts or a lazy eye will never see clearly unless the condition is corrected in the first years of life. Both mice and humans that lack adequate social contact as babies and juveniles have permanent behavioral and cognitive deficits.



Plasticity potential. A newborn neuron in an adult mouse brain.

The critical periods that arise earliest in development govern senses such as sight, hearing, and balance. Later ones govern higher-order skills such as language acquisition and social interactions. Most critical periods occur during infancy and childhood, when the brain is still growing and producing new neurons. But more important than the new cells are the connections the neurons make with each other. Connections that receive reinforcement are strengthened and protected, for example, by the growth of myelin sheaths around axons. Connections that go unused are pruned back.

In recent years, evidence has mounted that the critical periods close not only because plasticity-driving signals decrease, but also because the brain begins to produce signals that limit new connections between cells. When scientists use genetic tricks to remove these brakes on brain plasticity in experimental mice, the critical periods last well into adulthood. That's encouraging to those who wish to improve plasticity in adult humans, says Carla Shatz, a neuroscientist at Stanford University in Palo Alto, California. Boosting the plasticity of an adult human brain may not require replacing a whole network of signals that turn on that flexibility, she suggests. "Just take away the brakes,"

she says, and the brain can perhaps recover its lost capabilities.

In lab animals, at least, that's possible: Researchers have bred mice that lack some of the various genes that act as plasticity brakes. When these so-called knockout mice lose sight in one of their eyes for a few days—the researchers suture it shut—their brains quickly compensate and reassign more area to the good eye, a process that resembles the plasticity seen in newborn brains. The mutant mice also recover from strokes better than control animals. And in several tests of neural function, they seem like supermice. On a rotarod, a kind of motor skills test for lab mice that resembles a log-rolling contest, the knockout animals "are like Olympians," Shatz says. She and her colleagues have done a range of behavioral

"A fully plastic brain is not very helpful. It learns everything but remembers nothing."

—GERD KEMPERMANN,
CENTER FOR REGENERATIVE
THERAPIES DRESDEN

tests on their knockout mice. So far, "they're really good at everything." That's certainly not the whole story, she adds: "There has to be some downside."

One likely disadvantage is that too much rewiring can lead to short circuits—in a brain, that could mean seizures. Indeed, those same knockout mice respond to a smaller dose of seizure-inducing drugs than typical mice. In humans, the result of unleashing brain plasticity might be epilepsy. Shatz notes that unexplained epilepsy is much more common in childhood—when the brain is more plastic in many areas—and some epilepsy patients eventually outgrow their disease. A newborn "has to learn things fast or it's not going to survive. It's worth the risk of instability. But it's kind of dangerous to learn that fast. Once the organism has acquired fundamental experiences, you slow it down a bit and put on these brakes," Shatz says. Closing critical periods may also provide a firm foundation for further brain development, says Brigitte Röder, a neuropsychologist at the University of Hamburg in Germany. "If you're always shaking the basement, you can't build a taller house," she says. Takao Hensch, a neuroscientist at Boston Children's Hospital, notes that missing plasticity brakes are suspects not only in epilepsy but also in schizophrenia and Alzheimer's disease.

Some evidence suggests that the brain's plasticity can be augmented without the danger posed by completely removing the brakes. Michael Merzenich, a neuroscientist and professor emeritus at the University of California, San Francisco, explores how certain kinds of sensory signals—mainly sound and touch—can rewire adult brains. He and his colleagues have shown that specially designed computer games can improve performance on memory and other cognitive tasks in both children and older adults, even months after the training stops. Research led by Daphne Bavelier, a neuroscientist at the University of Geneva in Switzerland, has shown that playing action video games, such as Medal of Honor, can improve vision and several kinds of cognitive skills.

The success of those games might be linked to the brain's reward and attention systems, Hensch says. Several of the molecules identified as plasticity brakes involve these pathways. Two drugs that enhance attention, fluoxetine (better known as Prozac) and Aricept, can lengthen or even reopen critical periods in experimental mice. Both drugs are now in clinical trials for reversing the effects of lazy eye in childhood, and

in one clinical trial fluoxetine helped stroke patients recover lost motor skills.

Fluoxetine also seems to influence another type of brain plasticity, the growth of new neurons throughout life in certain parts of the brain. Although most neurogenesis stops in childhood, two areas of the brain keep producing new neurons: the subventricular zone, which connects to the olfactory bulb; and the subgranular zone of the dentate gyrus, a part of the hippocampus. There are several ways to boost the production of new neurons in these regions; increased physical exercise and exposure to unfamiliar or complex environments are two clear neurogenesis enhancers. Fluoxetine and other antidepressants that act through the dopamine pathway also increase the neuronal birthrate and may keep the newborn neurons flexible longer.

What this ongoing production of neurons means for the brain is unclear, however. Although the rate of adult neurogenesis in an individual's brain is correlated with certain kinds of learning, the connection is not straightforward.

Some evidence points to the idea that in the dentate gyrus, the new neurons may aid the brain in adjusting to new environments, perhaps by helping the brain detect unfamiliar aspects of an otherwise familiar setting. Kempermann has proposed that adult neurogenesis might be an adaptation that has helped certain animals—mice and humans, for example—to adapt to and thrive in a wide variety of unstable ecological niches.

The new neurons “are an extreme form of plasticity,” says Fred Gage, a neuroscientist at the Salk Institute for Biological Studies in San Diego, California. He and his colleagues have found that the newborn cells seem to have their own critical period, lasting roughly 4 weeks, during which they are particularly excitable. (Recent studies suggest that fluoxetine might lengthen this period.) Gage speculates that the birth of neurons provides a continually fresh source of short-term critical periods for certain kinds of learning throughout life. The newborn neurons “are young kids that respond to everything,” he says. By the time one set of neurons has grown up and settled down, there's another set of cells ready to take their place.

Determining how those new neurons interact with the circuits already in place might help scientists better understand how the circuits are wired in the first place—and how to safely and efficiently rewire when needed.

—GRETCHEN VOGEL

Brain Teasers

The brain poses many more than just the five quandaries we've highlighted on these pages. Delve into any one of them and you'll soon run into another. Remembering the past (p. 30), for example, is a significant part of human experience, which raises one of the slipperiest questions in all of science: What is the biological basis of consciousness? (See *Science*, 1 July 2005, p. 79.) The elusive nature of that problem has convinced some researchers to stick to memory. “It's as close to consciousness as I can get and still look myself in the mirror in the morning,” quips Loren Frank, a neuroscientist at the University of California, San Francisco. Below are six more mysteries of the brain that any neuroscientist should be proud to tackle. —GREG MILLER

Star power. Researchers now realize that star-shaped astrocytes do more than just clean up after neurons. Recent studies find that they help shape synaptic connections in the developing brain, influence synaptic function throughout life, and may go haywire in a number of neuropsychiatric disorders. Given that astrocytes make up nearly half the cells in the human brain, we know too little about them.

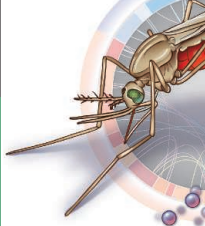
Uncharted territories. What the heck does the habenula do? Or how about the retrosplenial cortex? Some brain regions get all the love from neuroimagers (yes, we're talking about you, anterior cingulate), while others get ignored. There's still much to learn about these rarely studied regions, their anatomical connections, and their contributions to cognition and behavior.

Snooze fest. Why we sleep isn't just a mystery of the brain, but considering that it's the brain that switches animals into slumber mode, the organ must be at the heart of it. Do animals sleep simply to conserve energy and stay out of trouble in the dangerous dark? Or is sleep necessary, as some newer research suggests, to reset the brain to meet the challenges of a brand-new day? And what, if anything, does dreaming accomplish?

What's the code? Asking how information is encoded in the nervous system may be one step shy of asking how the brain works. But getting a better handle on how neural firing patterns—or is it which neurons are doing the firing?—represent information is crucial for understanding everything we do, including perception, memory, and decision-making.

Getting reconnected. The inability of the central nervous system of adult mammals to regenerate after injury is a vexing puzzle. Research with rodents has led to a better understanding of the cellular signals that put the brakes on such repair. But translating that work to people with spinal injuries remains an elusive goal.

Feeling immune. Many immune system proteins take on different roles in the brain, and immune responses are known or suspected contributors to a number of brain disorders. Yet scientists have only scratched the surface of how the immune system and nervous system interact. Recent findings that the gut microbiota may act through the immune system to influence the brain and behavior add another intriguing twist.



LETTERS

edited by Jennifer Sills

NextGenVOICES

Results: Big Ideas

In July, we asked young scientists to describe the one big idea in their field that they wish every non-scientist understood.

We heard from nearly 200 readers. A sample of the best responses can be found below. To allow for as many voices as possible, in some cases we have printed excerpts of longer submissions. To read the complete versions, as well as many more, go to <http://scim.ag/NextGen4Results>.

Submit Now: Experiments in Governing

Add your voice to *Science*! Our new NextGen VOICES survey is now open:

You've just been elected to your nation's highest office! In your inaugural address, announce the biggest challenge facing your country today and how you will use science to address it.

To submit, go to <http://scim.ag/NextGen5>

Deadline for submissions is 16 November. A selection of the best responses will be published in the 4 January 2013 issue of *Science*. Submissions should be 250 words or less. Anonymous submissions will not be considered. Please submit only once.

NextGen Speaks

RECENTLY, AT THE LONDON 2012 OLYMPIC Games, a famous Austrian swimmer found a creative explanation for his moderate success: He argued that intelligent athletes have a disadvantage in competitions because they tend to think too much. In an attempt to prove his claim, he pointed to an intelligent alpine skier who only won four World Cup races and an—in his opinion—rather unintelligent one who won more than 50. This might be an extreme example, but we encounter causal oversimplification and faulty generalizations so often in our daily lives that we barely notice them anymore. What makes matters worse is that politicians throughout the world—be it intentional or not—use logical fallacies as tools to justify unmoral actions and gain votes, inciting racism, sexism, and fear as collateral damage along the way. Their success is based on the lack of a basic understanding of logical fallacies and critical thinking. Much more than from a specific scientific concept, our society would benefit from knowledge of (and training in) scientific reasoning. The Austrian swimmer—although apparently hindered by his intelligence—managed to win 34 medals at major events throughout his very successful career.

ism, sexism, and fear as collateral damage along the way. Their success is based on the lack of a basic understanding of logical fallacies and critical thinking. Much more than from a specific scientific concept, our society would benefit from knowledge of (and training in) scientific reasoning. The Austrian swimmer—although apparently hindered by his intelligence—managed to win 34 medals at major events throughout his very successful career.

RUDOLF GRISS

Laboratory of Protein Engineering, École Polytechnique Fédérale de Lausanne, Lausanne, CH-1015, Switzerland. E-mail: rudolf.griss@epfl.ch

I WISH EVERY NON-SCIENTIST UNDERSTOOD [that].... [c]arbon markets are our best climate policy alternative because (through the availability of carbon offsets) they create options for lowest-cost climate change mitigation.... The fact that carbon offsetting can be good seems to have been lost in an ideological

logical debate about development justice, achieving emissions reductions in established industries, and the blind pursuit of governance rigor.... Scientific perspectives have been largely overlooked. We need offsets that contribute to genuine sustainable outcomes, but we also need to make progress on climate change mitigation, and offsets enable a more economically palatable transition.

PAUL DARGUSCH

School of Geography Planning and Environmental Management, University of Queensland, Brisbane, QLD 4072, Australia. E-mail: p.dargusch@uq.edu.au

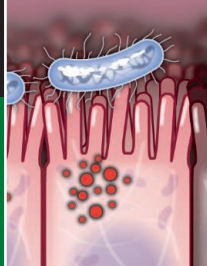
I AM AN ENVIRONMENTAL TOXICOLOGIST, and I wish that people understood the concepts of hazard and risk and the differences between the two. Chemicals are hazardous—even things we might think of as benign or “natural”—but they only cause a problem when they reach a concentration that is associated with a hazard. Risk [is a measure of both] hazard and exposure. I am frustrated by news and social media outlets that incite fear of “hazardous chemicals” in the general public without ever considering the level to which exposure is occurring.

KATHERINE COADY

The Dow Chemical Company, Midland, MI 48674, USA. E-mail: ktcoady05@gmail.com

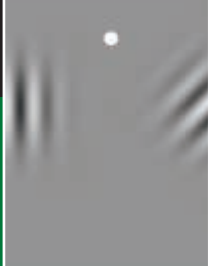
...NUMEROUS RECENT EXAMPLES OF RAPID phenotypic evolution in wild populations, occurring over the span of just a few generations, have taught modern biologists that evolutionary change is something we can often observe directly. Yet the average non-scientist (perhaps even the average non-biologist) thinks that evolution can only





Microbiota and cancer

52



Neurobiology Prize Essay

58



...THE IDEA OF EVER-increasing complexity is rooted in a strong adherence to the old Aristotelean world view of hierarchical classification of living things, which still permeates our everyday thinking. But in this case, it is time to let it go. The past two decades provided plenty of evidence that the endless variety of forms surrounding us is the product of just a handful of signaling pathways and gene regulatory modules, which are used iteratively over and over during development. Just as the imaginative combination of a limited number of Lego bricks can create a dazzling array of forms, so can changes in the regulation of key genes create new pigmentation patterns, new appendages or new behaviors. If people came to terms with this concept, most likely they would accept more readily our place in nature as just one branch on the Tree of Life....

MÁTÉ VARGA

Department of Genetics, Eötvös Loránd University, Budapest, H-1117, Hungary. E-mail: m.varga@ucl.ac.uk

crawl through the millennia at a pace that is slower than anything humans can easily imagine. This mindset allows many to safely ignore evolution, or, worse, reject it entirely.... [T]he misunderstanding that evolution occurs primarily on a geologic time scale has impeded societal endeavors such as human medicine, harvest of natural resources, and conservation efforts. But times are changing. Physicians are updating antibiotic dosage suggestions to curb the evolution of drug-resistant pathogens. Fisheries management agencies are applying life history theory to effectively manage wild stock. Conservation groups are realizing the importance of conserving genetic variation in threatened species....

A public grasp of one of the biggest ideas in contemporary evolutionary biology is within reach.

STEPHEN P. DE LISLE

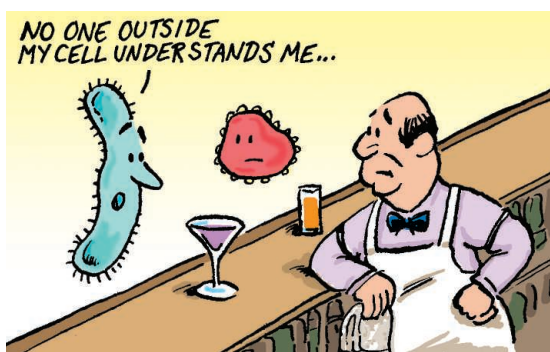
Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, ON M5S 3B2, Canada. E-mail: s.delisle@utoronto.ca

[I WISH EVERY NON-SCIENTIST UNDERSTOOD] the immunology underlying vaccines. It frustrates me to no end that available, effective inventions to combat disease aren't always used due to media reporting and mass hysteria. If the public and media networks fully understood the science behind vaccines, preventable deaths could be avoided.

ADITI HALDER

University of Queensland, St. Lucia, QLD 4067, Australia. E-mail: aditi.halder@uqconnect.edu.au

I WISH THE PUBLIC UNDERSTOOD THE ENDOSymbiotic theory. In realizing that mitochondria have a radically different genome than



what exists in our nuclear DNA, one has to grapple with a startling idea: Life as we know it would not exist without these small organelles, once mere foreign bodies inside our single-celled ancestors. For all our arrogance, humans are not independent from other life on this planet; the story of the natural world is not only about the struggle of individual species but about the relationships between them. Understanding our interconnectedness provides plenty of food for thought—and thoughtful citizens make thoughtful stewards.

JILLIAN WALKER

Department of Biological Sciences, California State Polytechnic University, Pomona, CA 91768, USA. E-mail: jswalker@csupomona.edu

I WORK IN THE FIELD of medical genetics and genetic counseling, an area where the contact with the non-scientific public is very intense. It would be great if we could better explain concepts related to risk of recurrence, recessive inheritance, and de novo mutations.... Sometimes it is very difficult to alleviate the sense of guilt that parents of malformed children experience....

EDUARDO PREUSSER DE MATTOS

Department of Genetics, Genetics and Molecular Biology Graduate Program, Federal University of Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, 91501-970, Brazil. E-mail: eduardo.mattos@ufrgs.br

[I WISH NON-SCIENTISTS understood] the idea that one of the biggest threats to public health is the rise in the drug-resistant pathogens. Due to increased global trade and travels, these pathogens can spread easily. Our global research system is necessary and timely. We never know when local problems and solutions may become global, and every part of the world has a contribution to make.

PATRICK KOBINA ARTHUR

Department of Biochemistry, Cell and Molecular Biology, University of Ghana, Legon-Accra, Ghana. E-mail: parthur@ug.edu.gh

...I WORK WITH DIFFERENT SPECIES OF fungi, many of which are pathogens.... I wish more people, non-scientists as well as some prominent scientists, understood that organisms that cause disease are defined as pathogens only in relation to the organisms they affect.... Pests causing infestations and epidemics are usually an integral part of the ecosystem. Often, we overlook the fact that diseases occurring at a larger scale are caused by an ecosystem imbalance....

[M]any times, imbalances are caused by anthropogenic factors and are indicators of our own short-sighted harmful activities that lead to medical and

environmental catastrophes....[I]f more people adopted a less anthropocentric perception of their existence, they would be freer to plan a sustainable future in the long term with research efforts and funding perhaps shifting toward...biodiversity and conservation.

LJERKA LAH

Laboratory for Molecular Biology and Nanobiotechnology, National Institute of Chemistry, Ljubljana, SI-1000, Slovenia. E-mail: ljerka.lah@ki.si

I AM IN SCIENCE POLICY AND I WISH EVERYONE had a better understanding that science itself is not politicized or inherently supportive of one party or another. Too many people, I think, believe that science serves only a particular viewpoint or political argument. In reality, science is the underlying foundation on which to make policy decisions that may or may not hinge on the science itself. With a better understanding of that, I hope people would not be as quick to criticize scientific reports and the scientists who produce them based solely on the conclusions.

ANISH GOEL

Technology Policy and Geopolitical Affairs, The Boeing Company, Seattle, WA 98101, USA. E-mail: anish.goel@boeing.com

...A CLEAR UNDERSTANDING OF PHARMACOGENOMICS would lead to safer prescriptions, reduced risk of adverse side effects, minimized healthcare cost, and improved clinical outcomes. In addition, a better understanding of this concept will encourage the public to participate in clinical trials in order to bridge the knowledge gap among clinicians, scientists, and the non-medical community. This will pave the way for the advancement of medicine and improvement of standard practice.

MICHAEL O. BACLIG

Research and Biotechnology Division, St. Luke's Medical Center, Quezon City, 1102, Philippines. E-mail: mobaclig@stluke.com.ph

[I WISH NON-SCIENTISTS UNDERSTOOD] THE concept of gene-environment interaction that could predispose us to the progression of certain diseases. Diseases are often multifaceted

in nature, and studying merely genetics or simple in vitro models is not sufficient in recapitulating the environmental factors that we are exposed to across the years.... If everyone can take a small step to mitigate possible deleterious gene-environment interactions by altering their lifestyles, we can potentially reduce our healthcare costs even without discovering novel treatments to delay or reverse the progression of the diseases plaguing the developed world.

BRYCE TAN

Yong Loo Lin School of Medicine, National University of Singapore, 119077, Singapore. E-mail: brycetant03@hotmail.com

UNCERTAINTY. IT DOES NOT MEAN I DON'T know. It is quantifiable. It is understandable. Communication between non-scientists and scientists would improve greatly if non-scientists understood scientific uncertainty. I work between the fields of environmental engineering and ecology

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FOCUS ON CAREERS

Neurodegenerative and Neuropsychiatric Disorders Research:

A Cerebral Career Choice

In This Issue

As people live longer, the incidence of age-related diseases, such as Alzheimer's disease (AD), will also increase. This potential for increased incidence of AD, as well as other neurodegenerative diseases such as Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS), introduces a critical need for medical breakthroughs that prevent or slow the progression of these diseases. Likewise, neuropsychiatric disorders such as depression and schizophrenia contribute to a substantial proportion of disability among both younger and older individuals. As a result, the field of neuroscience holds a wealth of career opportunities for graduate students and postdoctoral scientists now and in the decades to come.

See full story on page 142.

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in interdisciplinary research. My work links to sensitive issues such as climate change and environmental pollution. We are scientists, not fortune-tellers or psychics reading into the future. No, we cannot predict every exact detail what will happen because of human impacts on the Earth system. But, using scientific observations combined with sophisticated models, we can determine a range of what could happen and what most likely will happen....

SARAH M. ANDERSON

Nitrogen Systems: Policy-Oriented Interdisciplinary Research and Education-IGERT and School of Biological Sciences, Washington State University, Pullman, WA 99164, USA. E-mail: sarah.anderson2@email.wsu.edu

animals....Improper use and overuse of antibiotics are the major reasons for the increase in multi-drug-resistant bacterial strains. Bacteria may gain sovereignty again over humankind unless everyone stops overuse and misuse of antibiotics.

BISHNU P. MARASINI

Natural Product Research Laboratory, Nepal Academy of Science and Technology, Khumaltar, Lalitpur, Nepal. E-mail: bishnu.marasini@gmail.com

...AS A NEUROSCIENTIST WORKING ON learning and memory, I wish every non-scientist could appreciate



the beauty of our sophisticated neural circuits and understand that dramatic enhancement of human intelligence is optimistically possible....

CHUN-WAI MA

Department of Physiology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China. E-mail: cwma2010@hku.hk

...AS A PH.D. STUDENT, ONE OF THE MOST challenging—and sometimes discouraging—things for me to explain to a non-

scientist is why I chose to be a scientist, and why my research matters. The satisfaction and joy I have when accomplishing a hard-earned research project, adding valuable data to a long-term study, or discovering a new question to explore can be intangible to a non-scientist. I don't expect non-scientists to fully understand why I chose my career path, but the notion that science is a critical component to understanding our world, now more than ever, seems lost on many....

ELIZABETH PHILLIPS

School of Aquatic and Fishery Sciences, University of Washington, Seattle, WA 98195, USA. E-mail: emp11@uw.edu



[I WISH NON-SCIENTISTS WOULD HELP] stop overuse of antibiotics....[A]ntibiotic use for any kind of abnormal physiological condition is a common practice in developing countries like Nepal.



Even though we are not sick, we use antibiotics in our daily life, for example, cosmetics, shampoo, and meat from antibiotics-treated

Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the past 3 months or matters of general interest. Letters are not acknowledged upon receipt. Whether published in full or in part, Letters are subject to editing for clarity and space. Letters submitted, published, or posted elsewhere, in print or online, will be disqualified. To submit a Letter, go to www.submit2science.org.

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AGRICULTURE

Infusing Evolutionary Perspectives

Allison A. Snow

Taking a cue from Darwinian medicine (1), *Darwinian Agriculture* offers an engaging and bold explanation of why agricultural research must take better advantage of insights from evolutionary biology. A provocative and companionable author, agronomist R. Ford Denison (University of Minnesota) spent much of his career at the University of California, Davis. His writing varies from scientifically rigorous to casually anecdotal and from caustic to empathetic, as he describes the mistakes and successes of crop breeders, molecular biologists, and agroecologists who have taken on agriculture's great challenges. He has a deep interest in plant physiology, organic farming, and evolutionary biology, all of which contribute to the originality of his views on agriculture.

Denison argues that current efforts to improve agriculture are "risking failure" if core principles of Darwinian thinking are ignored. Noting that rapid evolution in weeds, pests, and pathogens is already well documented, he explores other terrain, offering fresh perspectives on how to improve crop breeding and agronomic practices. He examines food security and sustainable farming issues through an evolutionary lens, probing and searching for missed research opportunities that could enhance crop yields.

Among these is the development of crop traits that would not be favored by natural selection, such as Norman Borlaug's low-stature wheat and other crops that produce very high yields by allocating more resources to grain at the expense of height and competitive ability. Denison believes that additional opportunities for "reversing" past natural selection and thereby breeding more "cooperative" plants that boost crop yields could help alleviate global deficiencies in agricultural production. In other words, crop breeders should take note whenever traits that favor individual fitness under natural conditions—such as aggressive root growth or horizontally positioned leaves—can be jettisoned in favor of traits that improve overall yield by

enhancing the performance of the entire crop population.

Ever skeptical of biotech's silver bullets, Denison contends that using genetic engineering to enhance tradeoff-free photosynthetic efficiency or water-use efficiency may be extremely challenging because millions of years of natural selection may have already experimented with mutations that enhance plant fitness through these traits. Likewise, agroecologists who attempt to select for high grain yields from perennial grasses are unlikely to achieve what annual plants can offer because of underlying tradeoffs between perenniality and seed production. Denison argues that both genetic engineering and mimicry of natural ecosystems have the potential to improve agriculture, but only if evolutionary tradeoffs are understood and taken into account.

Broadly accessible, the book is perfect for discussion-based seminar courses and forward-thinking plant scientists. To fully appreciate the author's message, I recommend reading each chapter in sequence, taking time to pause and reflect on key concepts and examples before moving on. Denison covers many topics in crop ecology and evolutionary biology—touching on ideas of Thomas Malthus, Garrett Hardin, W. D. Hamilton, Richard Dawkins, and Wes Jackson, among others—and cites scores of peer-reviewed papers, several of which he critiques along the way. For example, he asks why a high-profile paper on genetically engineered drought tolerance in maize did not report available data from nondrought conditions, where yield tradeoffs may be lurking. He also questions the general applicability to agriculture of David Tilman's research showing that species-rich grasslands are more productive than monospecific plots (which had a lot of bare ground). Further, he exposes the confounding influence that plant spacing can have in yield trials of new cultivars. Jacob Weiner's studies of how tightly packed, evenly spaced planting patterns can reduce weed competition

are praised, as are Richard Sayre's efforts to engineer safer cassava that degrades its cyanide content during processing. I found Denison's high scientific standards and probing analysis of key studies to be commendable and balanced.

Calling himself a "troublemaker" for perpetually asking a lot of questions, Denison comes across as part genius and part dreamer. He is comfortable with an astounding diversity of topics, including how long the world's supply of phosphorus will last, why crop rotation works better than polyculture, how legumes impose "sanctions" on low-performing nitrogen-fixing bacteria in their roots (and how breeders might enhance this trait), and the extent to which past natural selection has already perfected many physiological traits that are relevant to agriculture. Yet he also likes to digress; not every reader will be fascinated by forays into the defensive chemicals produced by bacteria on fungus-farming ants. Nor will all readers be receptive to terms like the recurrent "nature's wisdom"



Less competitive. Shading from taller plants selects against the shorter wheat varieties of the Green Revolution that allocate more resources into seeds.

or the oft-repeated and obvious point that the structure and function of natural ecosystems have not evolved to maximize productivity (and therefore should not be imitated by agroecologists on this basis).

Nearly everyone will find something to argue about in this rambling, thought-provoking book, and I think that is part of the author's intent. By challenging the status quo and infusing Darwinian principles more centrally into agricultural research, Denison invites readers to test his hypotheses, build on them, and think more creatively about how to feed the world. This I applaud.

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Darwinian Agriculture
How Understanding
Evolution Can Improve
Agriculture

by R. Ford Denison

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Princeton, NJ, 2012.
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EVOLUTION

They've Kept On Keeping On

Emma Sherratt

Time travel is enticing to us; we relish the prospect of journeying back to see for ourselves how history actually happened. We have documents and archeological remains to tell our story, but imagine what more we might learn if some key characters in human history were still alive today.

In *Horseshoe Crabs and Velvet Worms*, Richard Fortey offers a colorful tale of extraordinary (and often unfamiliar) plants and animals that are ancient in origin and still found in present-day habitats. They are usually referred to as living fossils because they closely resemble organisms that lived hundreds of millions of years ago, known from their remains and impressions in rocks. Fortey explains that these “living fossils” may not be *the* ancestor, but they are survivors carrying a precious legacy of information from distant days and vanished worlds.” These organisms that “time has left behind” offer a novel approach to understanding the history of life.

However, Fortey prefers not to use the term “living fossil” because it insinuates they have not changed at all. “On this dynamic earth, nothing stays still: neither climate, nor life, nor the oceans and continents, although they all move to different rhythms.” That is, these organisms have changed over time, but they have also retained features that are perhaps primitive, because if something worked for their ancestors, why change it? Instead, “survivor” seems a much more fitting title, as it conjures up the image of a well-worn traveler that has endured all of the difficulties life has thrown at it.

The star survivor in the book is probably the horseshoe crab. There are four extant species of these prehistoric-looking, armored arthropods (not actually crabs), the most prolific living on the Atlantic and Gulf Coasts of North America and three less common ones

in Asia. Prehistoric indeed—today’s horseshoe crabs appear remarkably similar to fossil species that are more than 500 million years old. Over this time, the world’s biodiversity was intermittently decimated by five major extinction events, yet horseshoe crabs carried on.

Clearing these mass extinction hurdles is undoubtedly what makes the horseshoe crab story so impressive. And they are not alone. Coelacanths may be the most famous; they certainly warrant the epithet living fossil, as the fish were known from the rock record before being discovered alive off the coast of Africa. But perhaps the most alluring examples are the really ancient survivors, those that paved the way for all other organisms. Fortey takes readers to a beach on the west coast of Australia to introduce stromatolites, slimy cushion-shaped mounds, built by a living biofilm of cyanobacteria. These are the most ancient organic structures. “We should all be grateful to slime,” Fortey quips, because these bacteria changed the ancient atmosphere by adding oxygen. Fossil stromatolites date back to a staggering 3.5 billion years ago; their discovery transformed the way we understand early life on Earth.

By the means of such organic time machines, Fortey leads readers on a thrilling journey to exotic locations and others close to home, climbing up and down the tree of life to tell the stories of the many “messengers from deep geological time.” The tree analogy provides a map for his excursions. And there is no one better with whom to take this trip than Fortey—a paleontologist retired from the Natural History Museum in London and marvelous natural history writer. Often humorous, continuously captivating, the author demonstrates his distinctive approach to narrating stories of both the wonderful creatures and the people who dedicate their lives to studying them. Undoubtedly, the book will be as much at home on the coffee tables of natural history enthusiasts as it is on the shelves of science graduates.

Fortey completes the journey with an overview of life’s history through mass extinctions, hammering home the astonishing survivorship of these organisms. What is their secret? It might be adapting to change. Velvet worms, specialist predators of termites, appeared a long time before termites, so their lineage must have had the ability to exploit

new prey. It may also be physiology. Horseshoe crabs’ blood is special because it clots when exposed to bacteria, thus sealing off an infection. Living where few else can survive, such as the highly saline environment of stromatolites, is certainly a clever strategy. However, the survivors’ longevity may also simply be attributed to luck. And Fortey reminds us that the luck of these organisms and countless others could soon run out—because of the destruction and exploitation of contemporary ecosystems by one species, us.

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BROWSINGS

Relics: Travels in Nature’s Time Machine.

Piotr Naskrecki. University of Chicago Press, Chicago. 2011. 362 pp. \$45, £29. ISBN 9780226568706.

Zoologist and photographer Naskrecki provides an image-rich survey of organisms and habitats that have persisted with little change for up to hundreds of millions of years. These horseshoe crabs (*Limulus polyphemus*) spawning on a Delaware Bay beach are among the many intriguing “contemporary manifestations of Earth’s distant past” that he discusses.



CREDIT: © PIOTR NASCRECKI/COURTESY UNIVERSITY OF CHICAGO PRESS

Horseshoe Crabs and Velvet Worms

The Story of the Animals and Plants That Time Has Left Behind

by Richard Fortey

Knopf, New York, 2012.
374 pp. \$28.95.
ISBN 9780307263612.

Survivors

The Animals and Plants That Time Has Left Behind
HarperCollins, London, 2011.
£25. ISBN 9780007209866.

The reviewer is at the Department of Organismic and Evolutionary Biology and Museum of Comparative Zoology, Harvard University, Cambridge, MA 02138, USA. E-mail: emmasherratt@fas.harvard.edu

ENERGY

Deploying Off-Grid Technology to Eradicate Energy Poverty

Benjamin K. Sovacool

In 2009, about 1.4 billion people lived without electricity, and 2.7 billion depended on wood, charcoal, and dung for domestic energy needs (1–5). This lack of access to modern energy limits income generation, blunts efforts to escape poverty (6), affects the health of women and children (7), and contributes to global deforestation and climate change (8). Small-scale renewable energy technologies and distributed energy options, such as diesel generators and improved cookstoves, offer rural households modern energy services (6). Programs involving governments, businesses, nonprofit organizations, banks, and community cooperatives have expanded access to these technologies and their services in recent years.

But technology alone is insufficient, and in deploying it, we face pernicious barriers. Drawing from scholarship in community governance and common pool resources (9–13), as well as field research in 10 Asian countries (see the table), this article offers “design principles” or “ideal conditions” for overcoming many of these barriers. As one village leader told the author, “classically, energy planners have seen the access question as one involving ‘givers’ and ‘takers’: the utility giving electricity or donors giving technology, and the consumers taking it. This completely places the energy services provider and consumer into a false dichotomy.” This dichotomy can lead to project failure, as seen in Malaysia, India, Indonesia, and Papua New Guinea (see the supplementary materials).

Electric utilities defer expanding access to underserved or poor areas because such expansion is believed to mitigate commercial profit (14). Public-sector actors can be stymied by their inability to implement or finance projects and are always under pressure to satisfy other urgent public needs. National planners may hesitate to promote off-grid renewable energy projects, because the technology must be imported or they may want to look for “free money” through international donations (15). Government agencies have prioritized expanding access

for urban, rather than rural, areas, and they suffer rapid turnover in staff, due in part to uncompetitive pay and unstable political climates (16).

Prioritize High-Impact Actions

To overcome these sorts of obstacles, planners, retailers, and implementers should select “low-hanging fruit” first, achieving maximum impact on expanding access to modern energy services with minimal effort. Widely accepted in energy and climate policy (17, 18), this idea is not always recognized in energy development circles, partly because of poor education of staff in best practices, as well as limited investment in pilot projects and evaluation. Successful efforts should strive to recognize behavioral plasticity—attitudes influencing acceptance or rejection of a technology—alongside sheer technical potential (10). When structured to emphasize maximum penetration at least cost, measurable benefits can exceed their expense. Evaluations of Nepal’s Rural Energy Development Programme (REDP), for example, have documented as much as \$8 in benefits per household for every \$1.40 expended (19).

Enable Sufficient Financial Incentives

Effective programs almost always offer financial assistance to overcome the first cost hurdle related to off-grid energy technology—another idea accepted in energy policy literature but sometimes forgotten among practitioners who either have large enough budgets to

Projects must account for social and behavioral factors influencing renewable energy technology adoption.

give technology away at no cost or who operate in countries that lack robust financing or microfinancing sectors (20, 21). Microcredit, such as small low-interest loans, is used in Bangladesh to purchase cookstoves, solar home systems, and biogas units. In Laos, Provincial Electricity Supply Companies (PESCOs) own and lease solar home systems for a weekly fee. Cost-sharing from communities can lower expenses. Microhydro projects in Nepal and Sri Lanka asked for voluntary land donations for construction of canals, penstocks, power houses, and distribution lines. It also required villagers to contribute labor and civil works. Proper emphasis of these interventions is on affordability rather than installed capacity.

Distribute Reliable Information

Foreign technical experts and bankers design and implement most energy development programs, and they do not always have reliable information about domestic attitudes and local markets. Yet, valid information on energy technologies and delivery programs must circulate between credible sources and people, including energy end-users themselves. This can occur through formal programmatic demonstration efforts and/or informal social networks. In the cases examined here, marketing and promotion involved publication and distribution of catalogs; informational brochures; product displays; Web sites; and advertisements in print, radio, or on television. In Bangladesh, Grameen Shakti conducted large demonstrations of solar and biogas devices and employees made door-to-door visits to familiarize communities with technology. REDPs in China and Nepal, the Renewable Energy for Rural Access Project (REAP) in Mongolia, and the Energy Services Delivery Project (ESDP) in Sri Lanka did “road shows” where experts demonstrated technologies in rural areas.

Maintain Quality Assurance

Prefeasibility studies are important before a project commences as are rigorously setting and monitoring technological standards and after-sales service and maintenance. Yet these are often missing in existing programs because of limited resources and/or

TEN SMALL-SCALE, RENEWABLE ENERGY ASIAN CASE STUDIES*

Grameen Shakti, Bangladesh
Renewable Energy Development Project, China
Rural Electrification Program, Laos
Renewable Energy for Rural Access Project, Mongolia
Rural Energy Development Programme, Nepal
Energy Services Delivery Project, Sri Lanka
Village Energy Security Programme, India
Solar Home Systems Project, Indonesia
Small Renewable Energy Power Program, Malaysia
Teacher’s Solar Lighting Project, Papua New Guinea

*From Sep 2008 to June 2011, the author’s team conducted 441 interviews with 200 institutions, visited 90 renewable energy facilities, and held focus groups with 789 people from 43 communities. See Supplementary Online Materials (SOM) for details.

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a belief that managers already know what's best without the need for consultation or assistance. Successful programs frequently start with pilot programs before initiating full-scale projects and scaling up to greater production or distribution volumes. Independent evaluations of project performance, and/or penalties for noncompliance make a difference—as does setting realistic expectations. Grameen Shakti in Bangladesh closed offices and refused to pay participating organizations if they did not meet financial requirements. The REDP in China and REAP in Mongolia fined manufacturers millions of dollars for violating technical standards. The ESDP in Sri Lanka required that villagers interested in hydro schemes establish an electrical consumer society, solicit a project developer, fulfill administrative requirements, and obtain verification from a chartered engineer before they proceeded with loan applications. Successful programs emphasize after-sales service and maintenance, ensuring that rural populations or technicians care for systems. This can occur on the “supply side” through product guarantees, warranties, and assurances to buy back systems if communities are connected to the grid; or the “demand side” through training and free maintenance. The Mongolia REAP, for example, gave financial support for warranties and after-sales service call centers.

Encourage Nested Institutional Arrangements

Effective programs tend to receive external political support, including a dedicated or experienced implementing agency, integration with other policies and regulations, and a clear project champion. Approaches to secure such political support are complex and beyond this article's scope, but it is clear that planners may want to limit time and money spent on projects without such support because they may be hard-pressed to succeed and sustain themselves. The ESDP in Sri Lanka, for instance, was backed by public subsidies for electrification, as well as a renewable portfolio standard and tax credits; its advocates changed the constitution so that off-grid and grid-connected developers had legal rights to distribute and sell electricity.

Effective programs distribute responsibilities among diverse stakeholders, especially nonstate actors and community leaders. This allows for sharing of risks as well as “nested” organizational multiplicity, which can create checks and balances. Involvement of women's groups, multilateral donors, rural cooperatives, local government, manufacturers, nongovernmental organizations,

and consumers can increase performance of partnerships. Such input can accelerate feedback to correct errors and handle disputes and can improve legitimacy, because programs with broader support are less likely to be opposed, protested, or even attacked during civil wars and internal conflicts (22, 23). Typically, effective programs transcend multiple scales, from self-governance (e.g., community-formed microhydro functional groups in Nepal, cooperatives in Indonesia, and PESCOs in Laos) to national agencies (e.g., the Sustainable Energy Authority of Sri Lanka or Alternative Energy Promotion Center in Nepal) to international actors [e.g., the United Nations Development Programme (UNDP), World Bank, Global Environment Facility], making them “polycentric” (12, 13).

Build Human Capacity

Efficacious programs undertake capacity-building, including strengthening technical or managerial capacity of private and public firms and educating villagers and communities about productive energy uses. Planners can neglect to build capacity out of hubris, thinking that their institutions already have sufficient expertise; a desire to focus on the “simpler” act of deploying technology instead of the more difficult act of building human institutions; or, in rare cases, bias and corruption. Effective interventions have built private-sector capacity through basic research grants; manufacturing and production loans; and efforts to standardize, certify, and test technology. Programs can train staff at rural electricity companies, cooperatives, and manufacturers in tariff setting, metering, billing, revenue management, accounting, and auditing, as well as formulating business plans, advertising, and marketing. Other private-sector support can include staff recruitment and education, establishment of rural outlets, and expansion of inventory. Public-sector capacity can be improved through recruiting new staff, devising electrification and renewable energy deployment master plans, operating databases and new computer systems, and conducting feasibility studies and resource assessments. Assistance can enhance the ability to monitor and evaluate projects, report results, arrange bulk purchases, and create or upgrade government research and testing laboratories. Community-scale programs can educate households about energy and income generation (e.g., China's REDP offered nomadic herders tips on using solar electricity for lighting and to separate milk and cheese and for charging mobile phones).

Conclusion

Rural energy users must be viewed not as passive consumers but as active participants in energy projects. None of these principles or this shift in focus is necessarily new. Yet, energy development practitioners may be too busy, too determined to push a particularly “favorite” technology, or too proud to learn from each other and the academic literature to take them into account. In some cases, maldevelopment or negative impacts can occur if programs waste precious resources. Practitioners, and those interested in energy development, could start by shifting how they conceive of energy technology and program structure (table S4). No matter how dazzling and promising advances in energy science and technology may be, it will have an extremely limited effect in eradicating energy poverty unless programs take these principles into consideration.

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Supplementary Materials

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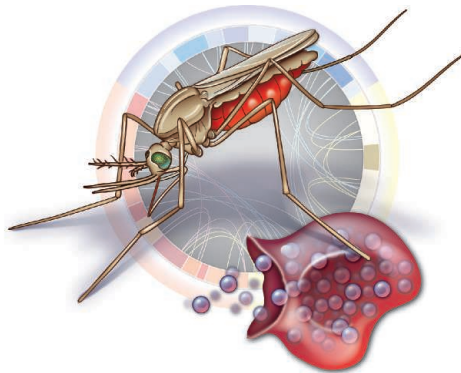
EPIDEMIOLOGY

Malaria in the Post-Genome Era

Brian Greenwood¹ and Seth Owusu-Agyei²

Ten years ago, the genome sequence of the mosquito *Anopheles gambiae*, the most important vector of malaria in Africa, and the genome sequence of *Plasmodium falciparum*, the most dangerous human malaria parasite, were published (1, 2). It was anticipated that these remarkable pieces of research heralded a bright future for malaria control. Has this happened? The genome projects have made some contribution to the development of new malaria tools, such as new vaccine candidates, but a decade is probably too short a period for the research to be translated into success in the field. Knowledge gained from the genome projects may contribute more substantially in the coming decade as efforts to control infection increasingly focus on development of new drugs, insecticides, and vaccines.

Mortality from malaria has fallen in the past decade, although there is disagreement over exact numbers. The World Health Organization (WHO) estimates 655,000 deaths from malaria per year (95% confidence limits of 537,000 to 907,000)—with about 90% of those deaths in Africa in 2010 (3)—whereas the Institute of Health Metrics and Evaluation reports 1,238,000 deaths per year (95% confidence limits of 929,000 to 1,685,000) because of inclusion of a greater number of deaths outside Africa and a large number of deaths among adult Africans (4). How can a difference of this magnitude be explained? In developing countries, many deaths occur at home and the cause can be ascertained only by a postmortem verbal questionnaire, which has poor specificity. Determinations of cause of death in hospitals may be incorrect because of inadequate diagnostic facilities. Thus, even complex statistical analyses of the global number of deaths from malaria must have wide confidence limits. Unfortunately, this factor is often ignored, so statements about the numbers of malaria deaths in response to environmental factors or control strategies may not give a true picture of the situation. This is damaging, and better methods are needed for monitoring progress in malaria control—a topic currently being



reviewed by WHO's recently established Malaria Policy Advisory Committee (5).

Data from some individual countries confirm the decline in deaths from malaria, but this has not been seen in all countries. In Africa, the decrease has been most marked on the continent's periphery, and there has been little change in the central areas where transmission is highest. The major reason for success is the increased political profile given to malaria as a result of enhanced advocacy by organizations such as the Roll Back Malaria partnership and the consequent provision of substantial funds for malaria control (about \$2 billion per year) by international and bilateral donor organizations. This has allowed the scale-up of existing, proven tools (conceived before the genomes were sequenced) for malaria control, such as simple rapid diagnostic tests, artemisinin-based combination therapies, artesunate for treating severe cases, long-lasting insecticide-treated bednets and, in some countries, indoor residual spraying of households with DDT or pyrethroid-based insecticides. Another reason for recent successes has been the increasing involvement of well-trained scientists from endemic countries in malaria research and control.

Despite these successes, challenges to control of malaria abound. Technical challenges come from the appearance of malaria parasites partially resistant to artemisinins in Southeast Asia (6), with a possibility of their spread to Africa, and increasing evidence that *A. gambiae* is becoming resistant to pyrethroids (7) threatens the effectiveness of long-lasting insecticide-treated bednets in Africa. These developments highlight the need to sustain research on discovering new antimalarial drugs and insecticides. So far, the genome projects have made only a lim-

New approaches to malaria control should include the development of tools that rely on genome-based information.

ited contribution to the development of new malaria control tools; new drugs and vaccines developed during the past decade have been based largely on existing technologies and targets. But knowledge gained from the genome projects should contribute more substantially in the future to creating more potent tools by providing, for example, a better understanding of drug (8) and insecticide resistance (9) and in identification of new candidate targets for drug and vaccine development. Indeed, new approaches that are being explored include vaccines against *A. gambiae*, the use of sterile male mosquitoes to control vector populations, genetic modification of mosquitoes to make them resistant to malaria infection, and infecting mosquitoes with engineered bacteria that inhibit parasite development. Many of these approaches rely on genome-based information. Such work is being undertaken by organizations such as the Medicines for Malaria Venture (10), the Innovative Vector Control Consortium (11), and individual academic groups.

Perhaps the greatest hope for controlling malaria is pinned on vaccination. Good progress is being made with RTS,S/AS01, a vaccine that provides about 50% protection against uncomplicated and severe malaria in children aged 5 to 17 months at the time of vaccination (12). Other promising vaccine candidates are in clinical trials (13). There is also renewed interest in malaria eradication (disappearance of the last malaria parasite)—a goal attempted during the 1950s and 1960s without success—and some readjustment of the research agenda is being undertaken accordingly with a focus on drugs and vaccines that block transmission (14). However, it is now widely accepted that unless there is some major new technical development that is currently not foreseen, such as a new way to interrupt mosquito behavior, this goal is many decades away. For now, optimum use of existing tools should allow the elimination of malaria (through interruption of local transmission) in an increasing number of countries on the margins of areas of highest transmission ("shrinking the map") (15) and a further reduction in the burden of malaria in areas where elimination is not yet feasible.

Several highly endemic countries in Africa are making sound economic progress and should be encouraged to take on increas-

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ing financial responsibility for their national malaria control programs. The international financial crisis and redirection of political and financial attention to other pressing global health problems such as malnutrition and family planning are potential threats to the investment needed to sustain and expand malaria control and research. Past experience shows the disastrous consequences of letting up on effective malaria control. It's time to reap the benefits of the mosquito and parasite

genomes to aggressively tackle this disease. Otherwise, this highly adaptable parasite and its vector will continue to outwit us and continue to kill millions.

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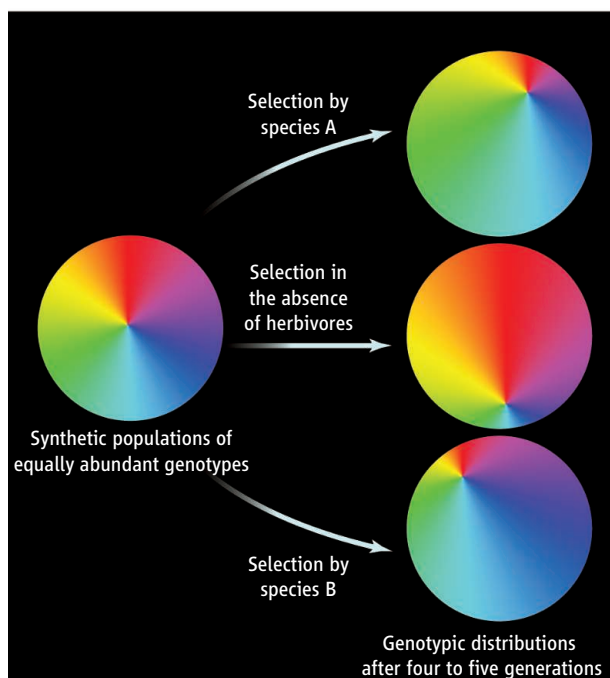
ECOLOGY

How Insect Herbivores Drive the Evolution of Plants

J. Daniel Hare

The most common biological interaction among species on Earth is that between plants and the insects that feed on them (1). Insect herbivores are thought to impose natural selection, which favors resistant plant genotypes and drives the evolutionary diversification of plant species. Two reports in this issue—by Züst *et al.* on page 116 (2) and Agrawal *et al.* on page 113 (3)—independently provide strong empirical evidence for the rapid evolution of plant traits that confer resistance to herbivores when herbivores are present but for the evolution of traits that confer increased competitive ability when herbivores are absent.

If resistance to insects benefits plants, then why are not all plants now resistant? Several answers to this long-standing question have been proposed. One is based on the assumptions that plant defenses are costly, that resistant genotypes are favored when the probability of insect damage is high, but that these genotypes pay a cost for resistance and are disfavored when the probability of herbi-



Divergent selection. Both Züst *et al.* and Agrawal *et al.* established synthetic plant populations consisting of equal proportions of plant genotypes and then observed changes in genotype frequencies after four or five generations of selection. The results show that natural selection favored different genotypes in the absence of herbivores rather than in their presence, and different genotypes in response to different herbivore species.

vore attack is low. In its simplest form, this hypothesis states that chemical resources obtained by plants can be allocated maximally either to growth and reproduction or to defense, but allocation to both processes cannot be maximized simultaneously (4). A second hypothesis is that defense polymorphisms are the result of variation in selection regimes due to variation in the size and mem-

The presence or absence of particular herbivore species influences which plant genotypes are favored by natural selection.

bership of herbivore communities at different locations (5). The two studies in this issue find strong evidence for variation in plant defense traits in response to particular herbivore species, but only partial support for the allocation hypothesis.

Züst *et al.* studied natural populations of *Arabidopsis thaliana* in Europe. They compared the geographic variation in the profiles of glucosinolates [a class of defensive chemical compounds in the plant family Brassicaceae (6)] with the distribution of two aphid species that feed only on brassicaceous plants. The frequency of the *GS-ELONG* gene, which determines the length of the carbon side chain on glucosinolate molecules, varied both with latitude and longitude. The aphid *Brevicoryne brassicae* predominated in areas where the plants produced glucosinolates with four-carbon side chains, whereas the aphid *Lipaphis erysimi* predominated in areas where plants produced glucosinolates with three-carbon side chains.

The authors next used a synthetic plant population consisting of genotypes that produce several different combinations of glucosinolates. After only five generations of selection, feeding by each aphid species selected for plant genotypes with glucosinolate profiles identical to those in the field locations where each aphid species predominated. By contrast, the plant genotype that predominated after five generations in a “no aphid” treatment produced relatively low levels of glucosinolates. This genotype was, however, eliminated from populations exposed to all aphid treatments (see the figure).

In a related but independent study, Agrawal *et al.* conducted a four-generation

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selection experiment in the field using *Oenothera biennis*, a plant species that consists of several asexual genotypes. Natural selection by ambient populations of insects favored plant genotypes with delayed flowering relative to genotypes on which insects were suppressed. Insects also selected for plant genotypes having different mixtures of ellagitannins, a group of hydrolyzable tannins produced by *O. biennis* implicated in chemical defense (7).

In the presence of insects, plant genotypes that produced relatively more ellagitannin trimers were favored over plants that produced relatively more ellagitannin dimers, even though all genotypes produced similar quantities of total hydrolyzable tannins. However, suppression of herbivorous insects on *Oenothera* also suppressed herbivores on competing plant species. As a result, suppression of insect herbivores indirectly selected for *Oenothera* genotypes that grew larger and competed better with other plant species (see the figure). In contrast to the allo-

cation hypothesis, the traits favoring growth were genetically independent of those favoring defense.

Both studies are consistent with earlier findings that herbivorous insects can impose natural selection on plant genotypes for defensive chemicals with specific structural attributes (8). For *Arabidopsis*, plant resistance to each aphid species was related to the length of the side chain of glucosinolates and not the total quantities of glucosinolates. For *Oenothera*, resistance was associated with selective synthesis of tannin trimers at the expense of tannin dimers. Both studies reinforce concerns that plant defense theories based on differential allocation of resources to broad classes of chemical compounds are of limited value (9).

Finally, the studies by Züst *et al.* and Agrawal *et al.* illustrate the importance of ecological context in predicting evolution. In the case of *Arabidopsis*, different specialist aphid species produced different evolutionary outcomes. In *Oenothera*, the evo-

lutionary outcome would surely have been different if the investigators had eliminated the effects of competition by maintaining their plots free of competing weeds, as other researchers often do. Because levels of herbivore pressure, the composition of herbivore communities, and levels of competition within plant populations all differ widely between habitats, evolution is expected to match plant genotype to habitat (8).

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DEVELOPMENTAL BIOLOGY

Intestinal Wound Healing Requires a Wnt Balancing Act

Terrence A. Barrett

Inflammatory bowel disease (IBD) encompasses a group of disorders of the colon and small intestine including Crohn's disease and ulcerative colitis. It affects roughly 396 per 100,000 persons worldwide (1) and in the United States is responsible for more than \$1.7 billion in overall health care costs. The chronic or recurrent inflammation associated with this disease causes severe damage to the epithelial lining of the intestine. On page 108 of this issue, Miyoshi *et al.* (2) present a model for wound healing in the intestinal tract that may have clinical relevance to mucosal repair in disorders of intestinal ulceration.

Within the epithelial lining of the small intestine and colon is a gland composed of subunits called intestinal crypts or crypts of Lieberkühn. After mucosal wounding, channels of epithelial cells move under exposed surfaces to begin to recreate normal crypt architecture. Epithelial proliferation at wound

edges is driven by the Wnt signaling pathway, particularly the canonical Wnts, which signal through β -catenin.

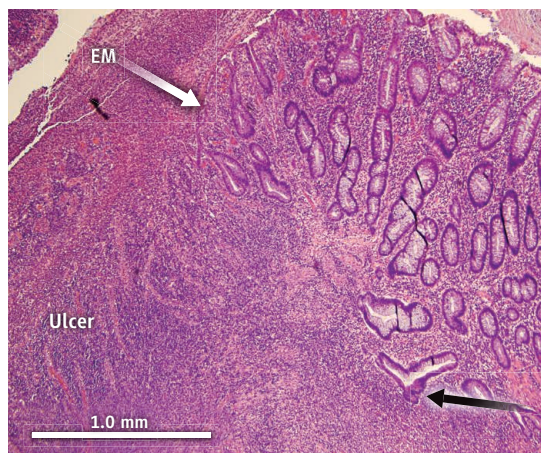
Miyoshi *et al.* have found that creation of new crypts requires release of noncanonical (β -catenin-independent) Wnt5a. Mesenchymal Wnt5a-secreting cells are derived from serosal mesothelial (WT1⁺) stem cells and appear to migrate into areas to participate in tissue repair. Wnt5a lowers epithelial prolif-

Control of wound healing via balancing signaling regulators may have implications for treating inflammatory bowel disease.

eration rates and induces epithelial channel invaginations or clefting under the wounded surface. Miyoshi *et al.* suggest that Wnt5a potentiates transforming growth factor- β (TGF- β) signaling (via Serpine1 and Smad3) to reduce epithelial proliferation and cause clefting of epithelial channels. Clefting alters the polarization of highly proliferative crypt structures at wound margins, allowing them to branch into new crypt units. The authors did

not detect Wnt5a-mediated inhibition of canonical Wnt signaling as reported by others (3). This inhibition, when observed, is context dependent (3), suggesting that non-canonical Wnt5a may affect canonical Wnt signaling in other mucosal healing processes in the intestine.

Wnt5a is needed for wound healing. Image of transmural ulcer from a Crohn's disease resection specimen showing an area of hyperproliferative branching crypts producing an epithelial monolayer (EM). An area of epithelial channel clefting is highlighted (dark arrow).



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Epithelial ulcerations that appear in IBD represent highly inflamed wounds caused by locally destructive immune responses (see the figure). A recent examination of Wnt family genes in IBD tissue found increased expression of Wnt5a localized to myofibroblasts (4). These data suggest that mechanisms for epithelial channel formation and clefting may be shared between wound repair and IBD ulcerations. Indeed, crypt branching is characteristic of chronic architectural distortion, a diagnostic feature of IBD. Given the high expression of Wnt5a in IBD tissue, it is possible that crypt branching may be related to the clefting described by Miyoshi *et al.*

Another potentially shared feature of IBD and wound healing may be the apparent dichotomy between noncanonical and canonical Wnt signaling. Both appear to be required for wound healing. Wnt/ β -catenin signaling is essential for maintaining the epithelial proliferative compartment (5). Analysis with a phosphospecific antibody for protein kinase A (PKA)/Akt-phosphorylated β -catenin at serine 552 (P- β -catenin⁵⁵²) showed increased nuclear P- β -catenin⁵⁵² in areas of chronic architectural distortion in IBD patients (6). Enhanced nuclear β -catenin production was also detected on aldehyde dehydrogenase-positive colonic stem cell populations in IBD tissue (7). Robust data also indicate that Wnt signaling is reduced overall in ulcerated mucosa. Wnt/ β -catenin signaling was reduced in a model of intestinal inflammation (acute dextran sulfate sodium-induced colitis), possibly due to induction of Wnt antagonist Dkk1 by interferon- γ (8). Data presented by Miyoshi *et al.* suggest that reduced Wnt signaling overall may also relate to noncanonical Wnt5a signaling released by stromal cells to aid ulcer healing and initiate new crypt structure formation. Thus, their study suggests that both enhanced canonical and noncanonical Wnt signaling may be required for efficient mucosal healing in patients with active IBD.

Mucosal healing is becoming a reliable clinical marker of recovery after IBD therapy as it is associated with durable clinical remission and reduced risk for future surgeries (9). The comparison between effects of prednisone and anti-tumor necrosis factor (anti-TNF) monoclonal antibody (mAb) therapies illustrate this point well. Whereas steroids reduce clinical symptoms, they heal mucosal ulcerations in as few as 29% of patients (10). Thus, most severe IBD patients experience recurrence within 1 year after steroid withdrawal (11). By contrast, TNF blockade through systemic humanized antibody delivery induces rapid mucosal healing (12). As

opposed to steroids, anti-TNF mAb treatment maintains remission and reduces the risk for surgery (13). The findings of Miyoshi *et al.* may help explain these observations, as they suggest that control of mucosal inflammation is critical for allowing repair mechanisms (such as those mediated by mesenchymal cells) to operate unhindered.

Steroids suppress a broad range of signaling pathways, including nuclear factor κ B (NF- κ B) signaling (14). NF- κ B induces a range of inflammatory molecules, such as chemokines that may be needed to recruit activated mesenchymal stem cells. Conversely, anti-TNF mAb therapy more specifically eliminates effector cells that induce mucosal damage (15). In the more targeted mAb approach, signaling pathways needed for ulcer healing (such as Wnt5a release) are not impaired. Although untested in IBD, it seems likely that the findings of Miyoshi *et al.* may allow better design of therapies in IBD that shut down destructive inflammatory

responses while permitting (or enhancing) optimal mucosal healing.

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CANCER

Bacteria Deliver a Genotoxic Hit

Robert F. Schwabe and Timothy C. Wang

Intestinal inflammation can promote colorectal cancer by altering the composition of the gut microbiota.

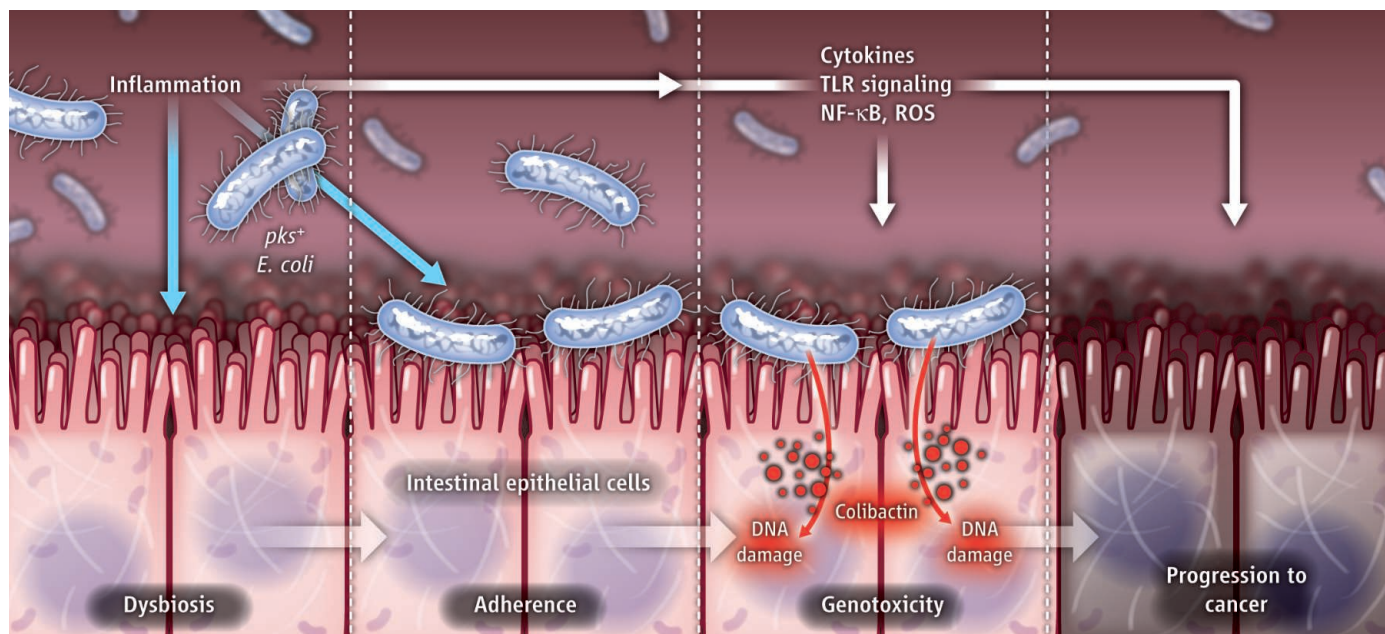
The adult human intestine contains up to 100 trillion bacteria that are beneficial to our metabolism and health (1). This microbiota is separated from the intestinal wall anatomically and functionally, thus preventing bacterial invasion and inflammatory responses. However, control mechanisms that ensure a symbiotic relationship between host and microbiota can fail in many diseases such as inflammatory bowel disease (2), complications of which may lead to colorectal cancer. On page 120 of this issue, Arthur *et al.* (3) provide evidence for a molecular link between the host immune response, the bacterial microbiota, and genotoxic events in inflammation-associated colorectal cancer.

The gut microbiota promotes carcinogenesis in the intestine (4) and other organs such as the stomach and liver (5–7). Although a specific pathogen such as the bacterium *Helicobacter pylori* may trigger gastric carcinogenesis (5), commensal bacteria contribute to chronic inflammation and more rapid cancer progression (7). Bacterial components

such as lipopolysaccharide also contribute to inflammation-driven carcinogenesis through Toll-like receptor signaling (6, 8, 9). However, most mouse models that investigate links between the gut microbiota, inflammation, and cancer use carcinogens such as azoxymethane that are not known contributors to inflammation-driven carcinogenesis in humans. It is therefore not well understood how changes in the gut microbiome and the host inflammatory response in patients with inflammatory bowel disease culminate in the genetic and epigenetic changes that ultimately lead to colorectal cancer.

To investigate changes in the gut microbial community that may contribute to colorectal cancer, Arthur *et al.* compared the microbiota between mice deficient in the anti-inflammatory cytokine interleukin-10 (IL-10), which are susceptible to colitis (colon inflammation) and azoxymethane-induced colorectal cancer, and wild-type mice that are resistant to these effects. By sequencing ribosomal RNA, the authors found an altered composition of the colonic-adherent microbiota in IL-10-deficient mice, with increases in the Proteobacteria phylum and the Entero-

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Bacteria, inflammation, and cancer. Chronic inflammation leads to dysbiosis and an expansion of genotoxic bacteria such as *pks*⁺ *E. coli* and their adherence to intestinal epithelial cells, resulting in a release of the genotoxin colibactin and DNA damage. These effects and the procarcinogenic effect of inflammatory pathways promote the development of dysplasia and cancer. TLR, Toll-like receptor; NF-κB, nuclear factor κB; ROS, reactive oxygen species.

bacteriaceae family [the latter includes *Escherichia coli*, a strain associated with human inflammatory bowel disease and colorectal cancer (10)]. Changes in the microbiota and the ~100-fold increase in *E. coli* were independent of azoxymethane treatment, suggesting that altered inflammation in the IL-10-deficient mice is alone responsible for the observed imbalance in intestinal bacteria (dysbiosis). Although IL-10-deficient mice colonized solely with either the *E. coli* strain NC101 or *Enterococcus faecalis* exhibited aggressive colitis and similar production of inflammatory cytokines, only the former developed invasive adenocarcinoma after azoxymethane treatment. These data support the idea that genotoxic actions (damage to host DNA) by *E. coli* exert specific carcinogenic effects independently of inflammation.

The NC101 *E. coli* strain produces the genotoxin colibactin (a polyketide synthase encoded by a genomic region called *pks*), which causes DNA double-strand breaks (11, 12). This strain was more prevalent in patients with inflammatory bowel disease (40%) and colorectal cancer (66.7%) than in healthy individuals (20.8%). Despite similar growth and proinflammatory effects, *pks*-deficient *E. coli* NC101 failed to induce DNA damage responses and caused a lower

tumor burden and less invasive cancer than wild-type *E. coli* NC101. Wild-type mice associated with *E. coli* NC101 also failed to develop colorectal cancer after azoxymethane treatment, suggesting that *pks*/colibactin-mediated effects require additional changes mediated by inflammation that is induced by a deficiency in IL-10. Arthur *et al.* suggest that inflammation not only increases *pks*-containing *E. coli* (*pks*⁺) but also promotes their adhesion to epithelia, a requirement for its genotoxicity (11, 12).

Inflammation is thought to induce or promote intestinal cancer through the effects of immune cells on epithelial cells, leading to oxidative stress, DNA damage, and cell turnover. However, the notion that chronic inflammation can lead to the accumulation of cancer-promoting bacteria begins to shift greater attention toward the microbiota (see the figure). This model is consistent with findings that a human colonic commensal, enterotoxigenic *Bacteroides fragilis*, may promote colon tumorigenesis through a specific toxin (13), and with studies suggesting a role for bacterial genotoxins in colonic dysplasia (14). To substantiate the findings of Arthur *et al.*, it will be crucial to determine whether *pks*⁺ bacteria promote colorectal cancer without addition of azoxymethane in longer-term animal studies. Colonizing mice with only *pks*⁺ *E. coli* is unphysiological and likely to overestimate effects of the polyketide synthase on carcinogenesis. Another limitation is the small number of patients investigated for *pks*⁺ *E. coli*. Confirmation in larger cohorts of patients with inflammatory bowel disease and colorectal cancer is needed to establish human relevance. There should

also be a more detailed metagenomic analysis that includes investigations on additional bacterial genotoxins and genotoxin-expressing bacterial species, given the substantial changes in other phyla such as Bacteroidetes in the IL-10-deficient mice. It would be relevant to determine whether the *pks* genomic region or its product colibactin is amenable to therapeutic approaches with specific chemical inhibitors.

The finding by Arthur *et al.* of a functional link between bacterial dysbiosis and carcinogenesis in chronic inflammation allows for a better understanding of the molecular mechanisms by which the bacterial microbiome promotes cancer. Together with the discovery of the carcinogenic effects of Toll-like receptor signaling (8, 9) and the mutagenic effect of bacterially induced reactive oxygen species (ROS) production (15), these advances point toward bacteria as new targets for cancer prevention or screening.

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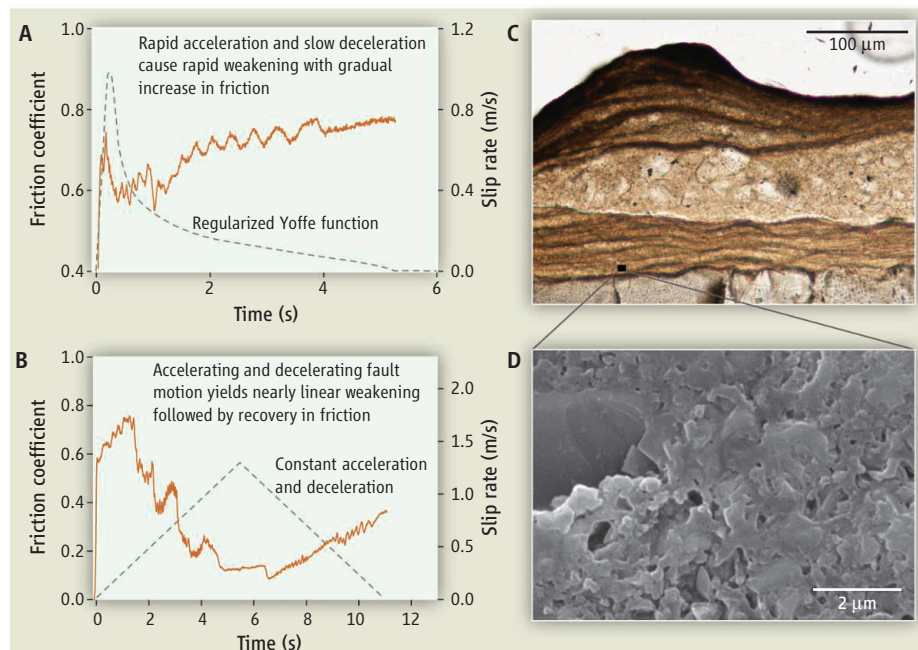
GEOPHYSICS

Earthquakes in the Lab

Toshihiko Shimamoto and Tetsuhiro Togo

Understanding how earthquakes of different sizes occur is one of the most challenging questions in fault and earthquake mechanics. On page 101 of this issue, Chang *et al.* (1) report the results of a carefully conducted experiment using a spinning flywheel attached to a high-velocity frictional testing machine to produce what they term an earthquake-like slip event. By changing the rate of revolution of the flywheel, the amount of kinetic energy transferred to the simulated fault in Sierra White granite or Kasota dolomite could be varied by about six orders of magnitude and could produce a series of frictional slips ranging from 0.003 to 4.6 m, corresponding to a moment magnitude range of $M_w = 4$ to 8 with respect to the range of fault displacements. The power relationship between energy input and displacement is similar to that found for natural earthquakes. Also, the slip produced by the flywheel is characterized by very rapid initial acceleration followed by gradual deceleration, somewhat similar to slip history recognized for natural earthquakes (2). Such experiments will arouse discussions about whether they are realistic proxies of natural earthquakes.

A rotary-shear high-velocity friction apparatus was developed in the early 1990s to reproduce seismic fault motion and measure frictional properties. Systematic studies have been conducted since then on frictional melting (3, 4), high-velocity weakening of gouge (fine particles constituting fault zones) without frictional melting (5), and mineral decomposition in gouge due to frictional heating (6). With respect to the flywheel experiments, it is noteworthy that different slip histories yield different frictional behaviors (7, 8). For example, two results are shown for Longmenshan fault gouge and compared with that obtained by Chang *et al.* (see the figure). Slip history expressed by the regularized Yoffe function (2, 8) yields slip weakening with a smaller characteristic distance followed by pronounced strength recovery (orange curve in panel A). Accelerating and decelerating slip history produces nearly linear weak-



Slip simulation. Frictional behavior of Longmenshan fault gouge and slip zone structures in quartz gouge. (A and B) Results from high-velocity friction experiments with slip histories expressed by the regularized Yoffe function (2, 8) and by constant acceleration and deceleration, respectively. The Longmenshan fault caused the 2008 Wenchuan earthquake, and gouge was collected at a surface outcrop in Hongkou, Sichuan, China. (C) Layered structures are overlapped slip zones in quartz gouge deformed at a slip rate of 1.3 m/s and a normal stress of 3.1 MPa (12). (D) Scanning electron micrographs [enlargement of the rectangular portion in (C)] show that quartz grains in gouge are welded.

ening followed by strengthening (orange curve in panel B). Both results are different from frictional behavior at constant slip rate, characterized by nearly exponential slip weakening from peak friction to steady-state friction (5, 9). The frictional behavior produced by the flywheel experiments is similar to that for slip histories expressed by the regularized Yoffe function, although the decelerating part is different from this function (compare panel A with figure 2 of Chang *et al.*). It is likely that rapid acceleration with slower deceleration leads to similar frictional behavior. Thus, Chang *et al.* raise the question of how the flywheel experiments can reproduce earthquake-like slip. This cannot be answered unless a constitutive law connecting low- to high-velocity friction is established and earthquake generation processes are solved on the basis of that law. The establishment of constitutive laws that can predict frictional properties with arbitrary slip history still remains a challenging task in fault mechanics.

Friction experiments using a flywheel-based apparatus can be used to simulate natural earthquakes.

Another problem relevant to the work of Chang *et al.* is that of powder lubrication as a weakening mechanism (10, 11). The exact weakening mechanism is unclear, although rolling friction of nanoparticles has been suggested (10). If grain rolling is important, however, an obvious question is raised: Why does dramatic gouge weakening occur only at high slip rates? Chang *et al.* propose enhanced gouge generation and powder lubrication as important mechanisms of slip weakening during the flywheel experiments.

Recent high-velocity experiments on quartz and Nojima fault gouge (12, 13) have demonstrated that sintering or welding of gouge particles in slip zones occurs at high slip rates as a result of frictional heating. Gouge experiments in the past few years have revealed that peculiar overlapped structures of slip zones form at high slip rates (see the figure, panel C). Such structures normally do not form at subseismic slip rates, and it is now thought that formation of welded grains (see the figure, panel D) makes slip zones

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strong, resulting in the shift of slip zone and deformation of existing slip zones forming complex structures such as overlapped slip zones. Formation of welded grains will suppress the powder lubrication; hence, the stage of slip at which welded grains form becomes an important question in evaluating the interpretation of Chang *et al.*

Gouge welding may shed light on the long-standing question in structural geology of how the fault gouge zone grows with increasing fault displacement. In a fault zone, gouge is weak and fault slip occurs in a gouge zone. The gouge zone then need

not grow once it becomes thick enough to accommodate fault displacement. Welding of gouge during seismic fault motion may give a mechanism for the growth of the gouge zone and may play important roles in the evolution of fault zone structures. Integrated studies on natural faults, laboratory experiments (like those of Chang *et al.*), and earthquake modeling will help to further delineate the characteristics of seismogenic fault motion.

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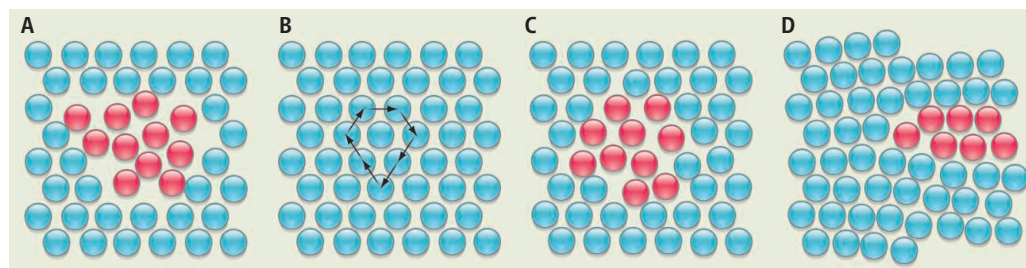
MATERIALS SCIENCE

Melting Colloidal Crystals from the Inside Out

Eric R. Weeks

Consider an ice cube melting in a glass of water. Ice cubes and any other solid objects almost always melt from their outer surface inward, and this scenario is well understood by physicists. What is less common, less well understood, but more intriguing is the case when a solid melts from inside outward (see the figure, panel A). Experimentally, this might be achieved by focusing an intense laser pulse inside a solid sample to locally superheat it. However, in such experiments, the sample melts extremely quickly, and it is difficult to determine exactly how the melting proceeds. On page 87 of this issue, Wang *et al.* (1) report the creative use of colloids as a model system to study the internal melting of samples in slow motion and with high magnification. They find that large agitations are the main driver of sample melting, rather than the formation of crystalline defects, contradicting some theoretical expectations.

Colloids are small, solid particles suspended in a liquid: Milk, blood, and paint are common examples. In 1986, Pusey and van Meegen demonstrated that colloids composed of identical hard spherical particles can order into crystalline phases at high particle concentrations (2). These experiments were enabled by the ability to synthesize polymer



Scenarios of melting. (A) A melted liquid patch (red) in the interior of a crystal is illustrated. (B) A possible loop rearrangement is shown where particles move as indicated by the arrows. (C) A group of particles (red) move large distances from their crystalline locations but the overall crystalline structure is still maintained. (D) A disclination forms where one of the red rows of particles disappears.

particles all of nearly the same size. Collections of hard spheres that interact only through short-range repulsive forces have long been known to be useful models of some phase transitions (3).

The work of Pusey and van Meegen inspired many groups to use colloids to study phase transitions (4). In all cases, the thermal (Brownian) motion of the colloidal particles lets the particles rearrange and transition from one phase to another. The control parameter for these experiments is not temperature or pressure, as would be the case for studying a single-component molecular system, but the volume fraction ϕ of the solid particles. For example, an equilibrated sample with $\phi > 0.545$ is fully crystalline and begins to melt if ϕ is lowered below this value.

Unfortunately, it is difficult to tune ϕ experimentally in situ. Wang *et al.* solved that problem by using special particles made from a temperature-sensitive polymer (5) so

How crystals melt can be seen directly by studying size-tunable colloidal particles.

that the particle diameter can be decreased by 13% as the temperature is raised from 26° to 31°C (and thereby decreasing ϕ by 46%). They prepared their samples in the crystalline state (with $\phi > 0.545$) and then used a microscope both to focus light onto the sample to heat it and to observe the subsequent melting.

In this experiment, particles engaged in melting moved in a variety of distinct ways. First, before any melting had occurred, particles occasionally rearranged in “loop motions” where one particle moved over, taking its neighbor’s position, and in turn that neighbor moved into another’s position, with the final particle in the loop moving into the first particle’s position (see the figure, panel B). These sorts of loop motions have also been seen in simulations (6) and occurred in regions where the Brownian motion of the particles allowed them to move farther from their ideal crystalline positions.

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These unusually mobile particles were first predicted in 1910 by Lindemann, who conjectured that the sample would melt once atoms in a crystal could move more than some fraction of the crystal-lattice spacing (see the figure, panel C) (7, 8). In the colloidal experiment, melting occurred when colloidal particles moved more than 20% of the lattice spacing, similar to what has been seen in simulations (9). In particular, the sample had to nucleate a small pocket of highly mobile particles, and if this pocket exceeded a critical size, this region continued to grow until the sample entirely melted. This critical size is limited by surface tension between the liquid and the crystal; Wang *et al.* measured a surface tension similar to what previous simulations of spherical particles have found. The colloidal experiment also revealed something new: If the sample was sufficiently superheated, multiple small liquid regions formed and could coalesce. Upon coalescence, surface tension caused the merged regions to adopt a spherical shape quickly. For even more extreme superheating, the colloidal crystal rapidly melted everywhere simultaneously.

Notably, what was not seen in the colloidal experiments were any defects such as dislocations, vacancies, and interstitials. Dislocations are irregularities in the crystal structure (see the figure, panel D), and vacancies are lattice sites where a particle leaves its normal

position and becomes an interstitial particle, located between other particles, deforming the crystal. These defects could be caused by the additional energy added to melt the crystal, and defects have been seen as important precursors to melting in simulations (10, 11). However, none of these defects were spotted in observations of dozens of melting colloidal samples. This absence is unlikely to be a limitation of colloids as a model system, because dislocations have been seen experimentally before in colloidal crystals (12).

Given that the experiments report phenomena similar to some simulations (6, 9) and different from others (10, 11), a natural concern is how to reconcile the discrepancies. Wang *et al.* compare their experiments to simulations of perfectly hard spheres (which only repel each other when they come in contact), which is a natural comparison for colloidal particles (2). However, there is very likely some softness in the interaction between the colloidal particles used by Wang *et al.*, which allows them to expand or contract dramatically with slight changes in temperature. Also, the different simulations used varying particle interactions and heating protocols.

The simplest interpretation is that there are probably several ways that a crystal can melt from the inside out, and which behaviors are seen will depend on which crystal is melted. In this case, the most striking observations from

the colloidal experiments are the good agreement with known behaviors of hard spheres (such as their crystal-liquid surface tension) and the merging of liquid regions when more strongly superheated. Given recent advances in tuning colloidal particle shapes and interactions (13–15), future colloidal experiments should be able to make even stronger connections with simulations.

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BIOCHEMISTRY

A DNA Twist Diffuses and Hops

Maxim Y. Sheinin¹ and Michelle D. Wang^{1,2}

Perhaps the reader can remember the good old days of wired phones, their cords so prone to the absent-minded twisting that eventually produced a multitude of small coiled coils. Wired phones are a thing of the past, but their cords still serve as inspiration to those interested in the coiling process of DNA. The striking beauty of DNA coiling (aptly named supercoiling) was first illustrated when Vinograd *et al.* (1) discovered multiple intertwined loops in their electron microscope images of a circular DNA from the polyoma virus. These loops, also called plectonemes, can play an important

role in gene regulation by bringing together distant DNA elements, such as enhancers and promoters (2). On page 94 of this issue, van Loenhout *et al.* (3) use single-molecule techniques to uncover the rich dynamics of plectoneme formation and movement.

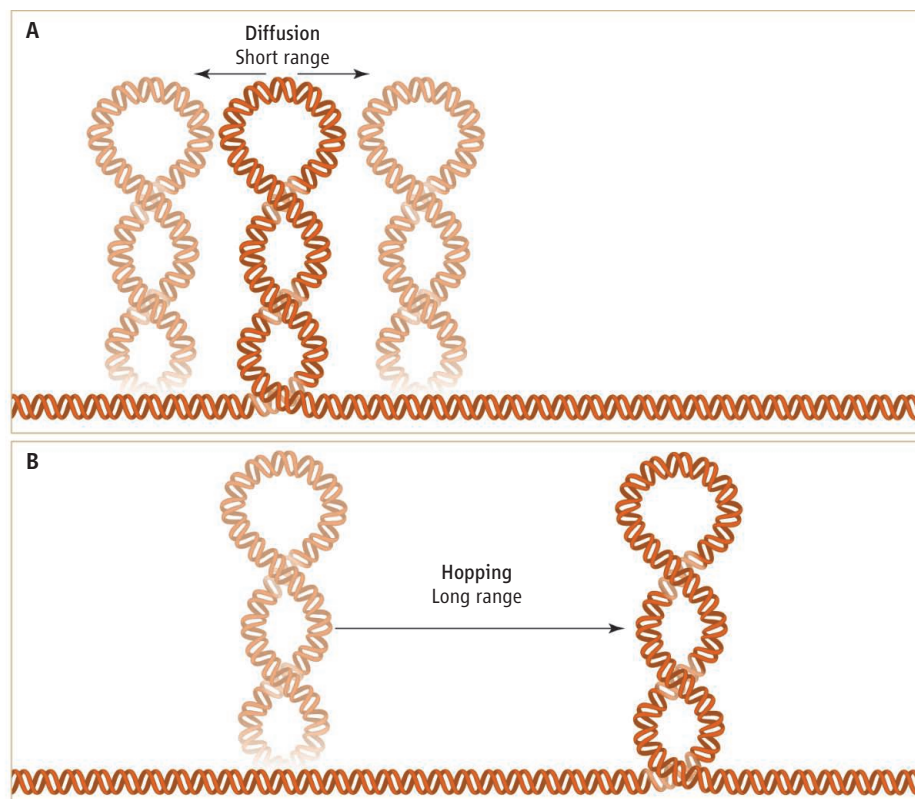
Electron microscope images of plectonemes have long captured scientists' imagination, but they provide only static snapshots of these DNA structures. Dynamic torsional manipulation of DNA was first demonstrated by Strick *et al.* in the mid-1990s using magnetic tweezers (4). In the experiment, a DNA molecule was torsionally constrained via multiple tags between a glass slide and a superparamagnetic bead. A pair of permanent magnets pulled the bead vertically toward the magnets until the magnetic force was balanced by the restoring elastic force

from the DNA. DNA was then supercoiled through the rotation of the bead. Plectoneme growth manifested itself as a steady decrease in the DNA length as the bead was turned to add twist to the DNA.

Subsequent experiments using an angular optical trap showed that the initial plectoneme formation is abrupt, as revealed by a sudden extension drop followed by a torque plateau (5). However, many questions remain. How many plectonemes coexist on a single DNA molecule? Do plectonemes remain at the same locations or move about dynamically? How and where do they nucleate, grow, shrink, and disappear?

To visually locate plectonemes along a 21-kilobase pair (kbp) DNA molecule sparsely labeled with fluorophores, van Loenhout *et al.* first supercoiled the DNA with

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Dynamics of DNA plectonemes. Plectonemes can undergo short-range diffusive motion along DNA (A). Sequence preferences—rather than hydrodynamic drag on a plectoneme, which is small—slow down the rate of such excursions. Plectonemes can also display hopping behavior (B), disappearing from one location and reappearing thousands of base pairs away within tens of milliseconds.

a pair of permanent magnets, as described above, and then used a side magnet to pull the supercoiled DNA tether sideways. The DNA molecule was visualized in this configuration using fluorescence. In this supercoiled DNA, bright fluorescent spots were detected. These spots reflected high local DNA density, indicative of the existence of plectonemes.

Under certain conditions, the researchers observed multiple plectonemes along the DNA. Their number varied from a single plectoneme at high force and salt concentration (3.2 pN, 300 mM NaCl) to about three at low force and salt concentration (0.4 pN, 20 mM NaCl). The authors attribute this trend to an interplay between the entropic gain provided by multiple plectoneme domains and the enthalpic cost due to the formation of initial plectonemic loops. The experimental results are in good qualitative agreement with two recent theoretical models (6, 7), underscoring the substantial progress that has been made in the quantitative understanding of DNA mechanics.

Plectoneme visualization was only the first step: Several seconds of imaging time permitted the authors to observe the long-coveted plectoneme dynamics. They found that each plectoneme diffused along the

DNA. However, the diffusion constant of an average plectoneme was much smaller than expected from a simple hydrodynamic model. This discrepancy is explained by the observation that the plectonemes were not evenly distributed along the DNA, but instead preferentially localized near certain positions. This suggests that diffusion takes place on a rugged energy landscape, modulated by the intrinsic curvature and bendability of the underlying DNA sequence. The authors found that roughness on the order of 1 to 2 $k_B T$ (where k_B is Boltzmann's constant and T is temperature) was sufficient to explain their diffusion data. This energy landscape roughness is of the same order of magnitude as has been estimated for the analogous energy barrier of plectoneme formation in DNA with intrinsic bends (8).

However, plectoneme dynamics turns out not to be limited to diffusion. Van Loenhout *et al.* report that plectonemes can hop, suddenly disappearing and rapidly reappearing thousands of base pairs away from their original locations. This unusual behavior appears to be one of the defining features of plectoneme dynamics, given that the mean lifetime of a plectoneme under most experimental conditions was less than 1 s.

Hopping could transplant the plectoneme several thousand base pairs within tens of milliseconds, whereas diffusional motion during that time was limited to a few hundred base pairs.

The visualization of plectonemes is a substantial experimental achievement. However, questions remain for further research. For example, van Loenhout *et al.* could detect plectonemes with a minimum size of ~ 0.5 kb, which is larger than the size of an initial plectonemic loop. Recent theoretical work (6) suggests that single loops can coexist with plectonemes; a more sensitive detection method is needed to test this hypothesis.

It would also be of interest to further investigate the hopping process. Van Loenhout *et al.* focus on hopping as formation of a new plectoneme concurrent with the disappearance of an existing one, but their data also hint at the redistribution of length among existing plectonemes. Further work will be required to determine if hopping is indeed a special case of length exchange among plectonemes.

The findings of van Loenhout *et al.* have important implications for processes that take place over DNA. The observation of preferential plectoneme localization suggests new ways in which DNA sequence can regulate genetic transactions. Indeed, certain DNA sequences may be designed to “pin down” plectonemes and thus bring neighboring regulatory DNA elements into close proximity. More research is required to identify the sequences that can localize plectonemes and analyze their distribution genome-wide. Plectoneme hopping, a dramatic long-range rearrangement of the DNA conformation on a millisecond time scale, could permit fast searching during DNA recombination or enhancer-activated gene expression. The next challenge will be to identify instances of plectoneme hopping in vivo and relate them to specific biological functions.

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EPENDORF

When Attention Wanders

Marlene R. Cohen

Everyone, from a child in a classroom to a driver on the road, has had the experience of having their thoughts meander far from the task at hand. Our minds wander when we rest (1–3) and about half the time we are engaged in an activity (4). A goal of my postdoctoral work with John Maunsell at Harvard Medical School was to understand how these internal fluctuations affect behavior. This knowledge is useful for determining the neural basis of internal mental states.

We measured the effect of fluctuations in one internal factor, visual attention, on perception. Attention allows observers to focus on the important locations (spatial attention) or features (feature attention) in a complex visual scene without looking at the attended object. For example, a baseball pitcher might focus his eyes on the catcher at home plate but also allocate part of his attention to the location around a runner at first base (spatial attention) or attend to the color of the opposing team's uniforms to keep track of runners at any location (feature attention).

Although attention has long been known

to affect perception, a detailed study of attentional fluctuations had been essentially unapproachable. Measuring fluctuations requires estimating a subject's attentional state at each moment. Attention improves a subject's average performance on perceptual tasks, but it is hard to know whether any particular mistake was caused by a lapse in attention or because the task was simply difficult.

Attention also affects parts of the brain that control vision: Attending to a particular location or feature causes the neurons in visual cortex that encode that location or feature to become more active (5–8). However, the responses of individual neurons cannot be used to estimate attention instantaneously because neural responses are noisy. It is impossible to separate out variability caused by changes in attention from variability that is inherent to the neuron.

The brain is thought to combat this noise by combining the responses of many neurons (9–12), so we used the same approach. We reasoned that if we recorded from many neurons simultaneously, we could tease apart the

Examining the neural process of visual attention helps decipher how the brain extracts important sensory information.

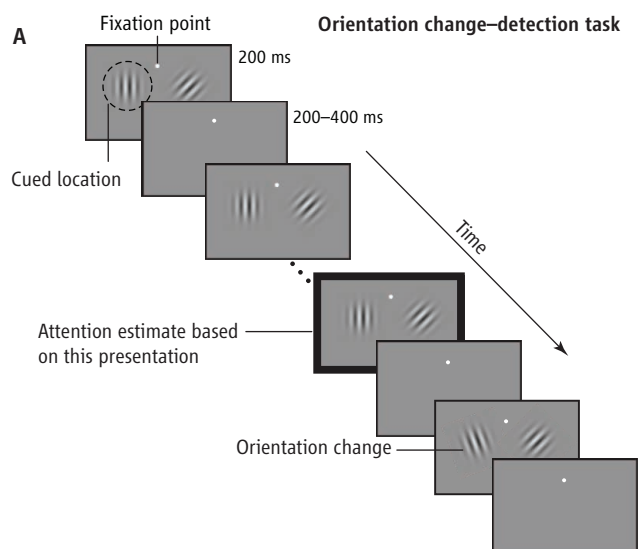
**eppendorf
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**PRIZE FOR
NEUROBIOLOGY**

Eppendorf and *Science* are pleased to present the prize-winning essay by Marlene Cohen, the 2012 winner of the Eppendorf and *Science* Prize for Neurobiology.

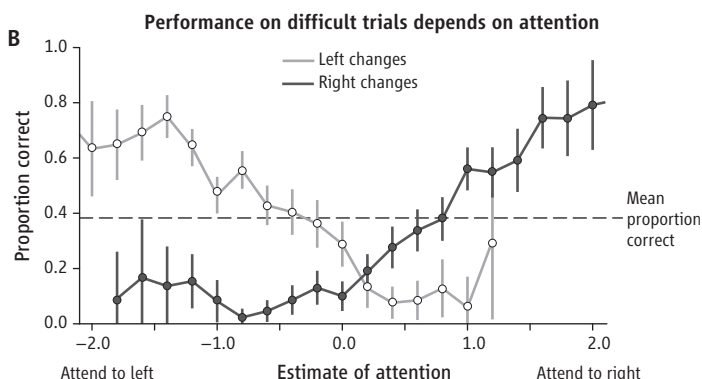
noise from responses that signaled changes in attention. After teaching monkeys a task that measured their ability to detect subtle changes in a visual stimulus, we simultaneously recorded the responses of about 80 neurons in cortical area V4, which encodes visual information and is affected by attention. The monkeys looked at a computer screen and a pair of striped stimuli flashed on and off [see the figure (A)]. At a random time, the orientation of one stimulus changed slightly. If the monkey correctly indicated that he noticed the change by looking at the changed stimulus, he was rewarded with a drop of juice.

To control their attention, we cued the animals as to which stimulus was most likely to change. When the monkeys knew to expect a change on the left, they shifted their attention to the left. We measured responses to the stimulus before the change [black outline in (A)] when the only difference from one trial to the next was the monkey's attention.

Single-neuron studies typically compare the neuron's average response across many trials in each attention condition (e.g., when the monkey expected a change in the left or the right stimulus). We modified this idea to estimate the monkey's attentional state



Change-detection task. (A) Rhesus monkeys were trained to perform a task that measured their ability to detect subtle visual changes. Two stimuli flashed on and off, and at a random time, the orientation of one of them changed. The monkeys were rewarded for looking at the stimulus that changed. Before each set of trials, the monkeys were cued to expect that one stimulus (the left one in this example) would



be more likely to change, which gave them incentive to pay attention to that stimulus. (B) The monkeys' proportion correct on the change-detection task is plotted as a function of the estimated location to which they allocated attention (which was calculated based on the responses of the neurons that were recorded while the monkey performed the task). [Modified from Cohen and Maunsell (2010) (22)]

at a single moment by comparing the combined response of the 80 neurons at a given moment to the average in each attention condition. We defined a measure of attention in which positive numbers signaled rightward attention and negative numbers indicated leftward attention [see the figure (B)].

Like humans (4), the monkeys' attention wandered, and our neuronal measure of attention varied greatly. Moreover, these attentional fluctuations profoundly affected how well they could do the task. When the measure showed strong attention to the left, the monkey was able to detect a subtle orientation change on that side about 70% of the time. However, when the measure showed that attention had drifted toward the right, he almost never detected that same change on the left [see the figure (B)]. This estimate of attention, based on just a few dozen of the ~100 billion neurons in the monkey's brain, allowed us to predict whether he was about to get the trial correct with about 80% accuracy.

Having a near-instantaneous measure of attention provided a new and powerful approach to investigate how attention affects performance and is controlled in the brain. To our surprise, whereas attention improved the monkeys' ability to detect subtle orientation changes, it worsened their performance when the change was very obvious (13), which suggests that strongly attending to one feature (e.g., vertical stripes) makes it more difficult to see a very different feature (e.g., horizontal stripes) (8, 14–21). Also, although the monkeys allocated attention to the two locations independently [they could attend to the left, right, both, or neither stimulus (22)], they nevertheless attended to a single feature in all locations (21). This suggests that spatial attention involves local groups of neurons whereas feature attention is coordinated throughout the brain.

Together, our results suggest that the baseball pitcher's spatial attention can wander from the catcher's mitt to first base or to both or neither location. When he attends to the color of the opposing team's uniform, however, he is sensitive to that color at all locations. But this improved sensitivity comes at a cost: When attending strongly, he might be completely unaware of his own manager running onto the field wearing a different color.

Our results show that when the mind wanders, so too do our perceptual abilities. Grasping the implications of variability in attention and other cognitive states on our basic abilities may be an important step toward understanding how our internal state affects our ability to interact with the outside world.

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2012 Grand Prize Winner



The author of the prize-winning essay, **Marlene Cohen**, is an assistant professor in the Department of Neuroscience and the Center for the Neural Basis of Cognition at the University of Pittsburgh. Dr. Cohen received her Ph.D. from Stanford University studying how interactions between neurons depend on how an animal is planning to use the sensory information they encode. Her postdoctoral research at Harvard Medical School used visual attention as a tool to understand which aspects of a cortical population code are most important. Her group at the University of Pittsburgh uses physiological, behavioral, and computational methods to study what information groups of neurons in visual cortex transmit to downstream areas and how variability in sensory neurons affects perception.

Finalists

Aryn Gittis, for her essay "Striatal interneurons: Causes or cures for movement disorders?" Dr. Gittis is an assistant professor in the Department of Biological Sciences and the Center for the Neural Basis of Cognition at Carnegie Mellon University. She received her Ph.D. from the University of California, San Diego, where she studied intrinsic firing mechanisms of vestibular nucleus neurons. In 2008, she became a postdoctoral fellow at the Gladstone Institute of Neurological Disease, where she studied inhibitory circuits involved in movement disorders such as Parkinson's disease and dystonia. Her laboratory uses electrophysiology, optogenetics, and anatomy to study how neural circuits in the basal ganglia control movement in health and disease. <http://scim.ag/Gittis>



Bertrand Coste, for his essay "The cellular feeling of pressure." Dr. Coste is a CNRS Research Scientist at the Research Center of Neurobiology-Neurophysiology of Marseilles, France. He received his Ph.D. in Neurosciences from the University of the Mediterranean Aix-Marseille II. In his doctoral work, he worked on pain sensitivity and investigated the modulation of nociceptive neuron excitability during inflammation. After receiving his Ph.D., he moved to The Scripps Research Institute in La Jolla, California, USA, where he was a postdoctoral fellow from 2007 to 2012. His work focused on the identification of molecular components involved in the transduction of mechanical forces into biological signals and led to the identification of a new family of ion channels. <http://scim.ag/Coste>



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Theory and Simulation in Neuroscience

Wulfram Gerstner,^{1*} Henning Sprekeler,^{2,3,4} Gustavo Deco^{5,6}

Modeling work in neuroscience can be classified using two different criteria. The first one is the complexity of the model, ranging from simplified conceptual models that are amenable to mathematical analysis to detailed models that require simulations in order to understand their properties. The second criterion is that of direction of workflow, which can be from microscopic to macroscopic scales (bottom-up) or from behavioral target functions to properties of components (top-down). We review the interaction of theory and simulation using examples of top-down and bottom-up studies and point to some current developments in the fields of computational and theoretical neuroscience.

Mathematical and computational approaches in neuroscience have a long tradition that can be followed back to early mathematical theories of perception (1, 2) and of current integration by a neuronal cell membrane (3). Hodgkin and Huxley combined their experiments with a mathematical description, which they used for simulations on one of the early computers (4). Hebb's ideas on assembly formation (5) inspired simulations on the largest computers available at that time (1956) (6). Since the 1980s, the field of theoretical and computational neuroscience has grown enormously (7).

Modern neuroscience methods requiring extensive training have led to a specialization of researchers, such that neuroscience today is fragmented into labs working on genes and molecules, on single-cell electrophysiology, on multineuron recordings, and on cognitive neuroscience and psychophysics, to name just a few. One of the central tasks of computational neuroscience is to bridge these different levels of description by simulation and mathematical theory. The bridge can be built in two different directions. Bottom-up models integrate what is known on a lower level (e.g., properties of ion channels) to explain phenomena observed on a higher level [e.g., generation of action potentials (4, 8–10)]. Top-down models, on the other hand, start with known cognitive functions of the brain (e.g., working memory), and deduce from these how components (e.g., neurons or groups of neurons)

should behave to achieve these functions. Influential examples of the top-down approach are theories of associative memory (11, 12), reinforcement learning (13, 14), and sparse coding (15, 16).

Bottom-up and top-down models can be studied either by mathematical theory (theoretical neuroscience) or by computer simulation (computational neuroscience). Theory has the advantage of providing a complete picture of the model behavior for all possible parameter settings, but analytical solutions are restricted to relatively simple models. The aim of theory is therefore to purify biological ideas to the bare minimum, so as to arrive at a “toy model” that crystallizes a concept in a set of mathematical equations that can be fully understood. Simulations, in contrast, can be applied to all models, simplified as well as complex ones, but they can only sample the model behavior for a limited set of parameters. If the relevant parameters were known, the whole or a major fraction of the brain could be simulated using known biophysical components—a prospect that has inspired recent large-scale simulation projects (17–21).

How can theory and simulation interact to contribute to our understanding of brain function? In this article, we illustrate the interaction with four case studies and outline future avenues for synergies between theory and simulation. Even though it forms an important subfield of computational neuroscience, the analysis of neuronal data (22–24) is not included in this review.

From Detailed to Abstract Models of Neural Activity: Bottom-Up Theory

The brain contains billions of neurons that communicate by short electrical pulses, called action potentials or spikes. Hodgkin and Huxley's description of neuronal action potentials (4) today provides a basis for standard simulator software (25, 26) and, more generally, a widely used framework for biophysical neuron models. In these models, each patch of cell membrane is described by its composition of ion channels, with specific

time constants and gating dynamics that control the momentary state (open or closed) of a channel (Fig. 1C).

By a series of mathematical steps and approximations, theory has sketched a systematic bottom-up path from such biophysical models of single neurons to macroscopic models of neural activity. In a first step, biophysical models of spike generation are reduced to integrate-and-fire models (27–32) where spikes occur whenever the membrane potential reaches the threshold (Fig. 1B). In the next abstraction step, the population activity $A(t)$ —defined as the total number of spikes emitted by a population of interconnected neurons in a short time window—is predicted from the properties of individual neurons (33–35) using mean-field methods known from physics: Because each neuron receives input from many others, it is sensitive only to their average activity (“mean field”) but not to the activity patterns of individual neurons. Instead of the spike-based interaction among thousands of neurons, network activity can therefore be described macroscopically as an interaction between different populations (33, 35, 36). Such macroscopic descriptions—known as population models, neural mass models, or, in the continuum limit, neural field models (Fig. 1A)—help researchers to gain an intuitive and more analytical understanding of the principal activity patterns in large networks.

Although the transition from microscopic to macroscopic scales relies on purely mathematical arguments, simulations are important to add aspects of biological realism (such as heterogeneity of neurons and connectivity, adaptation on slower time scales, and variability of input and receptive fields) that are difficult to treat mathematically. However, the theoretical concepts and the essence of the phenomena are often robust with respect to these aspects.

Decision-Making: Theory Combines Top-Down and Bottom-Up

Many times a day we are confronted with a decision between two alternatives (A or B), such as “Should I turn left or right?” Psychometric measures of performance and reaction times for two-alternative forced-choice decision-making paradigms can be fitted by a phenomenological drift-diffusion model (37). This model consists of a diffusion equation describing a random variable that accumulates noisy sensory evidence until it reaches one of two boundaries corresponding to a specific choice (Fig. 2A). Despite its success in explaining reaction time distributions, the drift-diffusion model suffers from a crucial disadvantage, namely the difficulty in assigning a biological meaning to the model parameters.

Recently, neurophysiological experiments have begun to reveal neuronal correlates of decision-making, in tasks involving visual patterns of moving random dots (41, 42) or vibrotactile (43) or auditory frequency comparison (44). Compu-

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tational neuroscience offers a framework to bridge the conceptual gap between the cellular and the behavioral level. Explicit simulations of microscopic models based on local networks with large numbers of spiking neurons can reproduce and explain both the neurophysiological and behavioral data (39, 45). These models describe the interactions between two groups of neurons coupled through mutually inhibitory connections (Fig. 2C). Suitable parameters are inferred by studying the dynamical regimes of the system and choosing parameters consistent with the experimental observations of decision behavior. Thus, the pure bottom-up path is complemented by the top-down insights of target functions that the network needs to achieve.

Theory has provided a way to identify the connection between the simulation-based biophysical level and the phenomenological drift-diffusion models that were used to quantify behavioral data in the past. This analysis first involves reducing the system of millions of differential equations to only two equations, by the mean-field techniques (33) discussed in the previous paragraph (see Fig. 1A). In this reduced system, different dynamical regimes can be studied by means of a bifurcation diagram (Fig. 2B), leading to a picture in which the dynamics of decision-making can be visualized as a ball rolling down into one of the minima of a multi-well energy landscape. Close to the bifurcation point (DD), finally, the dynamics can be further reduced to a nonlinear one-dimensional drift-diffusion model (46) (Fig. 2A).

Hebbian Assemblies and Associative Memories: Top-Down Concepts

The name of Hebb is attached to two different ideas that are intimately linked (5): the Hebbian cell assembly and the Hebbian learning rule. The former states that mental concepts are represented by the joint activation of groups of cells (assemblies). The latter postulates that these assemblies are formed by strengthening the connections between neurons that are “repeatedly and persistently active together.” These strong connections enable the network to perform an associative retrieval of memories (Fig. 3A): If a neuron that is part of the assembly does not receive an external stimulus sufficient to trigger

its firing, it can nonetheless be activated by lateral excitatory input from active partners in the assembly, such that eventually the stored activity pattern is completed and the full assembly—and thus the memory—is retrieved. Although Hebb wrote down his ideas in words, these soon inspired mathematical models of learning rules and large-scale simulation studies (6). Whereas

the simulations pointed to limitations of Hebb’s original ideas in practical applications, mathematical studies (11, 48, 49) cleared the first paths through the jungle of possible model configurations toward a working model of memory retrieval. In analogy to models of magnetic systems and spin glasses in statistical physics, the picture of memory retrieval as movement toward

a minimum in an energy landscape emerged (Fig. 3B), first in networks of binary units (12, 50) and later for rate models (51, 52). In these frameworks, important questions could be answered, such as that of memory capacity. Because each neuron can participate in many different assemblies, memory capacity is difficult to estimate by pure verbal reasoning. Theory shows that the number of different random patterns that can be stored scales linearly with the number of neurons (53): A network with 10,000 neurons can store about 1000 patterns, but if we double the number of neurons, we can store twice as many.

It took many steps from these abstract concepts to arrive at biologically plausible models of memory. Whereas the energy landscape analogy is restricted to a limited class of abstract models, the concept of memory retrieval as movement toward an attractor state (Fig. 3C) turned out to be robust with respect to model details. The top-down path (Fig. 3, C to E) of model development includes the transition from high-activity states to low-activity states (54); from networks with complete and reciprocal connectivity to ones with sparse and asymmetric connections (55); tuning of inhibition in sparsely active networks (56); transition from rate neurons to spiking neurons (34, 57, 58); addition of a spontaneous state at low firing rates (59, 60); homeostatic control of threshold or plasticity (47, 61); and further steps still to be taken, so as to solve the issue of online learning in associative memory networks (62). The above historical sketch shows the flow of ideas from top to bottom, by adding biological realism and details to well-understood abstract concepts. As research moves along this path, numerical simulations gain in importance, but theory remains the guiding principle. Given the technical challenges of detecting distributed but jointly active neuronal assemblies in the brain, it is not surprising that experimental evidence for associative memories as

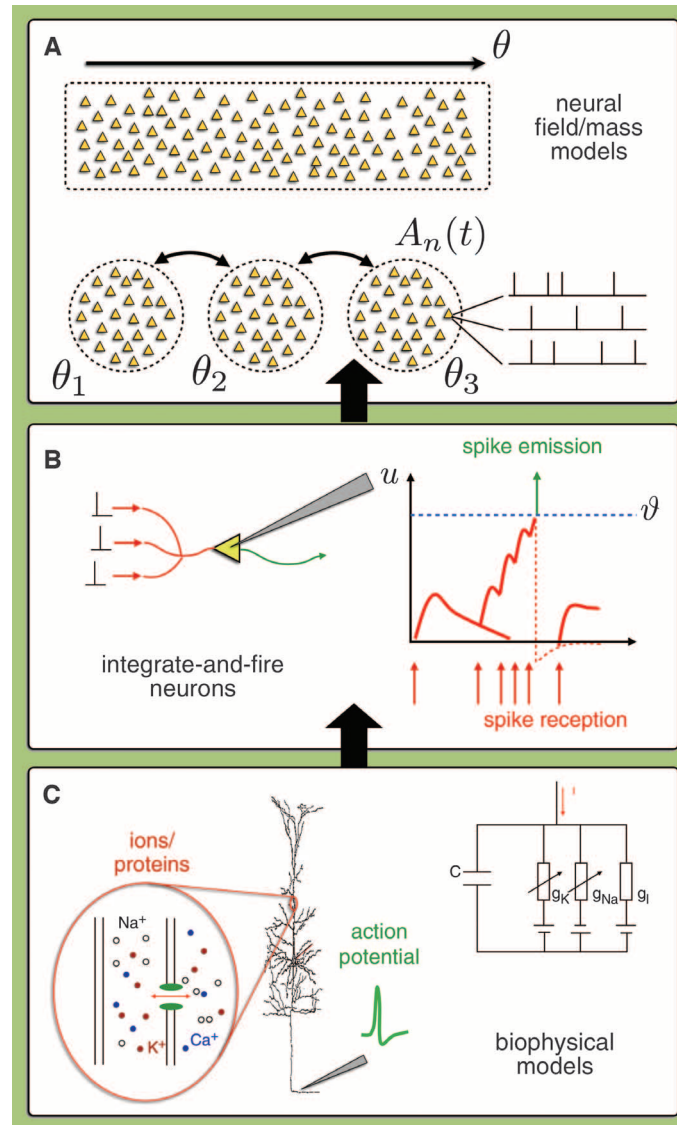


Fig. 1. Bottom-up abstraction scheme for neuronal modeling. **(A)** Neural mass model at the macroscopic scale. **(B)** Integrate-and-fire point neuron model. **(C)** Biophysical neuron model. The ion currents flowing through channels in the cell membrane (left) are characterized by an equivalent electrical circuit comprising a capacity C and a set of time-dependent conductances g , one for each channel type (right). These currents can generate action potentials (green). At the next level of abstraction **(B)**, action potentials are treated as formal events (“spikes”) generated whenever the membrane potential u (solid red line) crosses a threshold ϑ (dashed blue line). After each spike the voltage is reset (dashed red line). Arrivals of spikes from other neurons (red arrows) generate postsynaptic potentials. At the highest level of abstraction **(A)**, groups of neurons interact by their population activity $A_n(t)$ derived mathematically from neuronal parameters. In a continuum description (neural field model), different neuronal populations are characterized by their input characteristics such as the preferred orientation θ of a visual stimulus.

attractor states remains scarce and indirect: Neither invariant sensory representations as observed in individual high-level neurons (63) nor persistent activity during working memory tasks (64, 65) are sufficient to prove attractor dynamics, and the interpretation of multiunit recordings during remapping in the hippocampus as a signature of attractors (66) has been questioned (67), so that novel experimental approaches that enable large-scale recordings of complete neural ensembles (68–70) will be necessary.

Reward-Based Learning: From Behavior to Synapse

Hebbian assemblies play a role in models of decision-making (e.g., two populations representing choices A or B), but the assemblies are fixed and do not change. As such, these models describe static stimulus-response mappings that do not take into account that we can alter our decision strategies, that we can learn from experience. Theories of behavioral learning are an example for a top-down approach that has led from psy-

chology to models of synaptic plasticity, which go beyond a simple Hebbian learning rule.

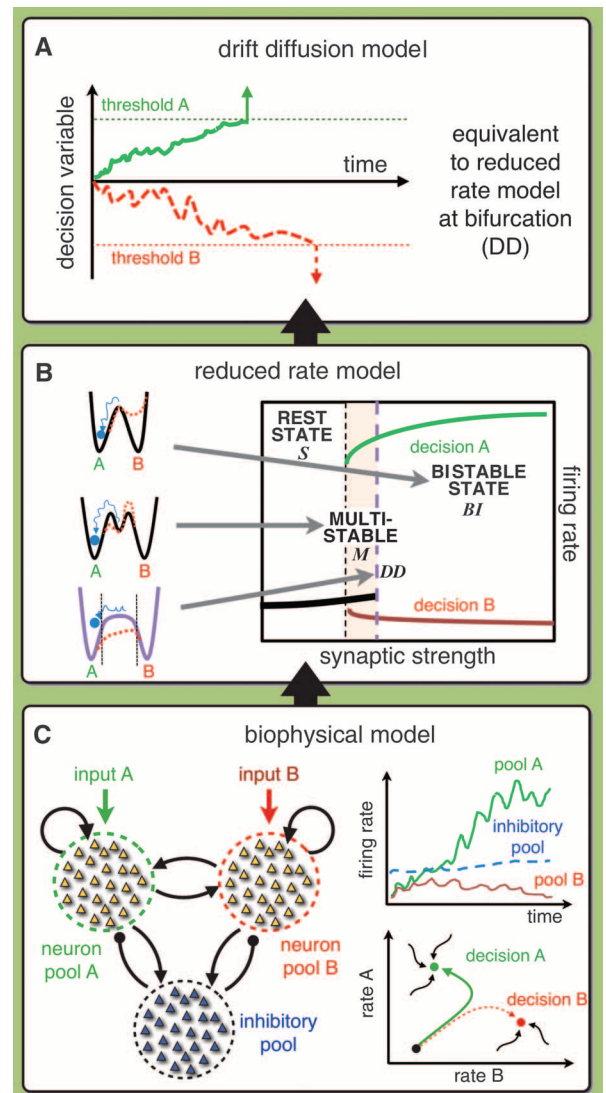
Models of behavioral learning date back to early experimental psychology. Thorndike's "law of effect" (71) stated that animals use behavioral alternatives more often when they were rewarded in the past. The mathematical theory of Rescorla and Wagner (13) extended this idea and suggested that it is not reward per se that drives learning, but the discrepancy between actual and expected outcome—an insight that is also essential in models of temporal difference learning (72). In the 1980s, this line of research on trial-and-error learning in animal psychology and artificial intelligence joined the parallel, mainly engineering-driven line of control theory (73) to form the large research field of reinforcement learning (74).

Although reinforcement learning often makes use of neural architectures (75, 76), its main focus is algorithmic. The renewed interest of computational and theoretical neuroscience in reward-based learning was triggered in the 1990s by two physiological findings. First, Schultz and col-

leagues discovered that dopamine neurons in the midbrain respond to unexpected rewards (77) with activity patterns that resemble the reward prediction error of temporal difference learning (14). Moreover, it was shown that synaptic plasticity, long hypothesized to form the neural basis of learning (78), is under dopaminergic control (79–81) and is thus modulated by reward prediction errors. These findings have led to the idea that the traditional Hebbian view of synaptic plasticity driven by pre- and postsynaptic activity must be augmented by reward prediction error as a third factor (80). The current tasks of computational neuroscience are to compare the learning rules suggested by the top-down approach with experimental data, and to generalize existing concepts in order to evaluate whether, and under what conditions, three-factor learning rules (82–84) of reward-modulated Hebbian synaptic plasticity can be useful at the macroscopic level of networks (85, 86) and behavior (87, 88).

Reinforcement learning is a textbook example for a synergistic interaction of theory

Fig. 2. Binary decision-making. **(A)** Behavioral level: Drift-diffusion model for reaction time experiments (37). A decision is taken (arrow) when the decision variable hits a threshold (first trial, green, choice A; second trial, red, choice B). **(B)** Mesoscopic level: Mathematical model of interacting populations. A decision can be visualized (left) as a ball moving down the energy landscape into one of the minima corresponding to A or B. The black lines in the energy landscape correspond to an unbalanced (50-50) free choice (i.e., no evidence in favor of any decision); the dotted red lines represent the cases with evidence for decision A. The three different landscapes correspond to different parameter regimes, indicated in the bifurcation diagram (right). In the multistable region (M), the spontaneous state is stable, but noise can cause a transition to a decision. In the bistable region (BI), a binary choice is enforced. At the bifurcation point (DD), the landscape around the spontaneous state is flat and the decision dynamics can be further reduced to the drift-diffusion process in (A). The vertical dashed line in the energy landscape indicates the "point of no return" and correspond to the decision thresholds in (A). **(C)** Multineuron spiking model (38, 39). Two pools of neurons (left) receive input representing evidence for choice option A or B, respectively, while shared inhibition leads to a competition between the pools. In the absence of stimuli, the system is spontaneously active, but if a stimulus is presented, the spontaneous state destabilizes and the dynamics evolve toward one of the two decision states (attractor states, bottom right). During a decision for option A, the firing rate of neurons in pool A increases (top right, green line). The mathematical model in (B) can be derived from the spiking model in (C) by mean-field methods (40). The parameter of the bifurcation diagram in (B) is the strength of excitatory self-interaction of neurons in (C).



and simulation. Most algorithms are based on mathematical theory, but without an actual implementation and simulation, it is difficult to predict how they perform under realistic conditions. The undesired increase in learning time with problem complexity observed in simulations of most algorithms poses challenges for the future. The solution could lie in efficient representations of actions (89) as well as environmental and bodily states (90), possibly adapted through Hebbian learning; place cells could serve here as an example (91, 92). Large-scale simulations will be key to evaluating whether neuronal representations across different brain areas are apt to turn complex reward-based learning tasks into simple ones, or whether we need a paradigm shift in reward-based learning.

Large-Scale Simulation Needs Theory

With the growth of computer power, the notion of large-scale simulation continues to change. In the 1950s, networks of 512 binary neurons were explored on the supercomputers of that time (6); both network size and biological realism increased in the 1980s to 9900 detailed model neurons (93). Current simulations of networks go up to 10^9 (94) or 10^{11} (95) integrate-and-fire neurons or 10^6 multicompartment integrate-and-fire models with synaptic plasticity (96).

The structure of neural networks, which is intrinsically distributed and parallel, lends itself to implementations on highly parallel computing devices [e.g., (97, 98)] and has triggered specialized “neuromorphic” design of silicon circuits (99, 100). Future implementations of large networks of integrate-and-fire neurons with synaptic plasticity on specialized parallel hardware (101–104) should run much faster than biological real time, opening the path toward rapid exploration of learning paradigms. Current large-scale implementations on general-purpose or specialized computing devices are feasibility studies suggesting that the simulation of neural networks of the size of real brains is possible. But before such simulations become a tool of brain science, major challenges need to be addressed—and this is where theory comes back into play.

First, theory supports simulations across multiple spatial scales. Even on the biggest computers it is impossible to simulate the whole brain at a molecular resolution. However,

in (for example) a study of the interaction of a drug with a synapse, the synapse could be simulated at the molecular level; the neuron on which the synapse sits could be simulated at the level of biophysical model neurons; the brain area in which the circuit is embedded could be simulated at the level of integrate-and-fire models;

and the remaining brain areas can be summarized in population equations that describe their mean activity. Theory provides the methods to systematically bridge the scales while ensuring the self-consistency of the model.

Second, theories guide simulations of learning. Because learning (at the behavioral level)

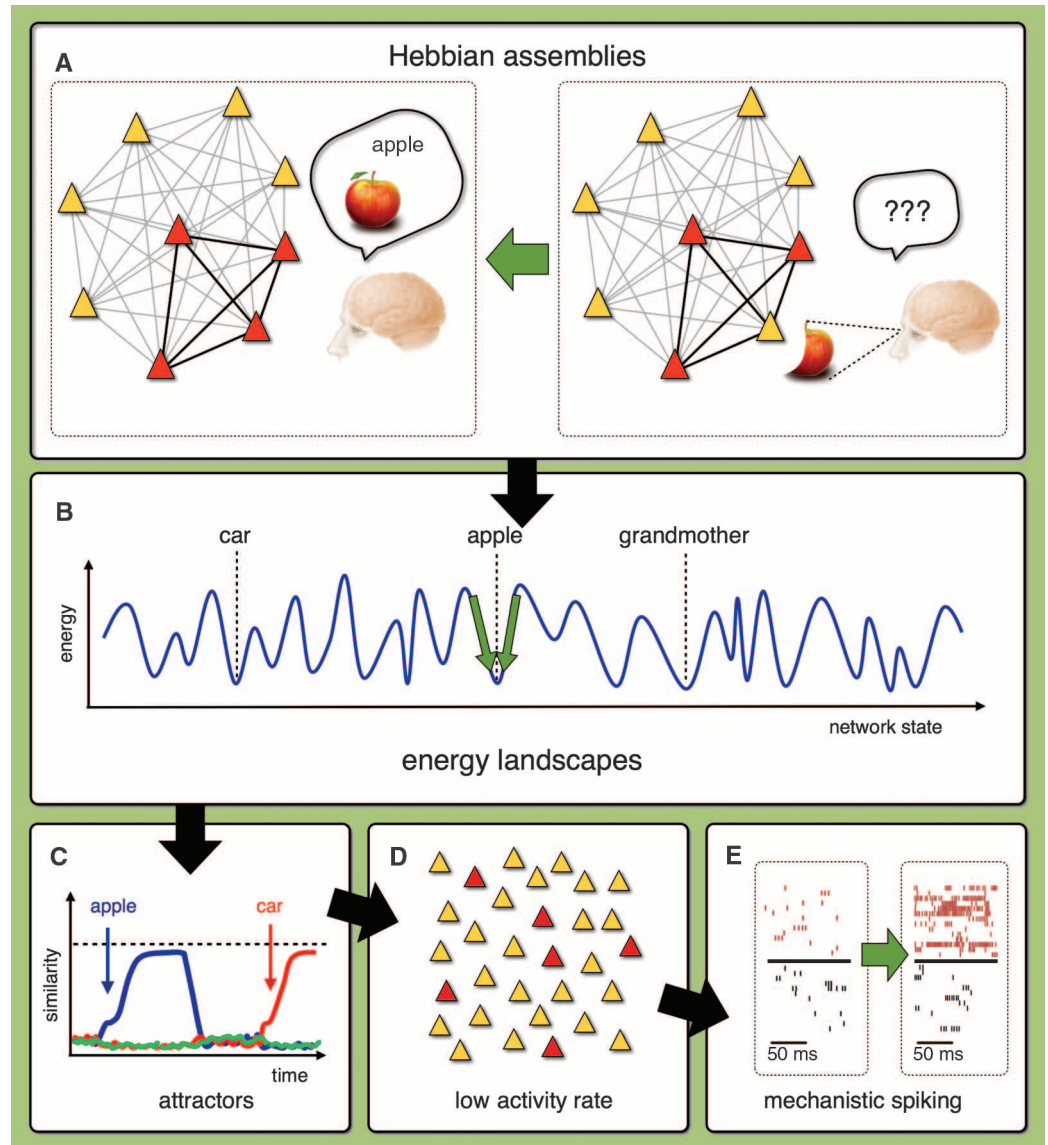


Fig. 3. Top-down evolution of ideas and models from Hebbian assemblies to associative memories. (A) Left: According to Hebb, neurons that are active together (red triangles) during a concept such as “apple” form a cell assembly with stronger excitatory connections with each other (thick lines) relative to connections to and from inactive neurons (yellow). Right: Partial information is sufficient to excite the inactive neuron of the assembly and retrieve the full concept (green arrow indicates flow of network dynamics). (B) The dynamics can be visualized in an energy landscape (energy as a function of network state) as a downward flow into the well corresponding to “apple”; other concepts (car, grandmother) correspond to other minima of the energy. (C) Similarity of the present network state with one of the assemblies. After a short presentation of partial information resembling the concept “apple,” the network moves toward an attractor state with high similarity (blue) to the corresponding assembly. After a reset, the network is ready to respond to a second stimulus (“car,” red). Similarity with other learned patterns (green) remains nearly unchanged during retrieval of one of the memories. The dashed horizontal line indicates maximal similarity, and therefore identity, with a stored assembly. (D) In a brainlike neural network, only few units have elevated firing rates (red). (E) Memory retrieval from partial information also works in networks of excitatory (red) and inhibitory (black) spiking neurons. In the spontaneous state (left), each neuron emits only a few spikes (vertical bars along a horizontal line); during memory retrieval, a few excitatory neurons fire many spikes (right). [Adapted from (47)]

and long-term plasticity of synapses (at the cellular level) evolve on the time scale of hours, experimental progress is slow and data to constrain synaptic plasticity rules are scarce. Even before a candidate plasticity rule is implemented in a simulation, theories allow us to predict, in some cases, that a given rule cannot work for the task at hand; that another rule should work, but might be slow; and that yet another rule should work perfectly if combined with some finely tuned homeostatic process, etc.

Third, regularization theories provide a framework for optimization of parameters. Even at the level of a single neuron, the number of parameters is huge because there are about 200 different types of ion channels (105), the density of which varies along the dendrite. Moreover, in a network of billions of neurons, each with thousands of connections, the number of parameters to specify the connectivity is daunting. How then, can experimental findings ever sufficiently constrain such detailed models?

A solution to this problem could be provided by regularization theory, which penalizes parameter settings that deviate from what is considered plausible. The challenge in the application of regularization to biologically detailed neural networks consists in designing appropriate regularization terms that summarize plausible prior assumptions in a transparent fashion. For example, ion channel distributions along a dendrite can be regularized by imposing plausible density profiles (10, 106) and penalizing the use of more than a few channels for any neuron (107). Connectivity can be regularized by penalizing deviations from simple “connectivity rules” (108, 109).

Fourth, theory drives understanding. It is extremely difficult to control a complex simulation if we do not have an intuition of the basic mechanisms at work. Mathematical abstraction forces us to express intuitions rigorously—to turn ideas into toy models. These not only make qualitative predictions of how a complex simulation should behave under changes of parameters, but also provide the understanding necessary to communicate ideas to others.

Theory Needs Simulations

Although theoretical concepts exist for some brain functions, many of our cognitive abilities still await neurally plausible models—for example, verbal and mathematical reasoning or representations of language and music. Will existing and future concepts developed in mathematical toy models be transferable to systems of the size of a brain with realistic input and output? We believe so, but extensions to large systems cannot currently be tested. In our opinion, mathematical toy models will continue to play a major role in guiding the way we think about neuroscience. However, initiatives for shared modular and reusable code and standardized simulator interfaces (110–113) are important; today the biggest problems addressed in computational neuroscience are often limited to the size of one Ph.D. project.

More generally, the community of theoretical and computational neuroscience would profit from a simulation environment where the ideas developed in the toy models could be tested on a larger scale, in a biologically plausible setting, and where the ideas arising in different communities and labs are finally connected to the bigger whole.

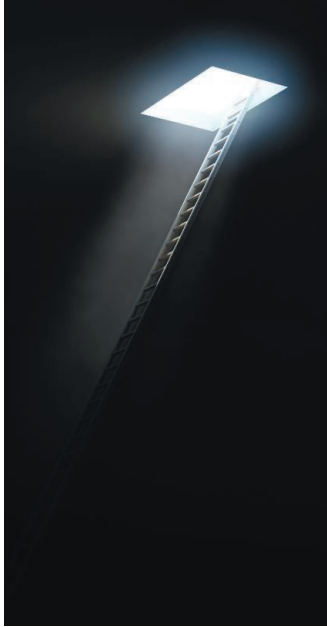
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INTRODUCTION

Defeating the Dementors

DEPRESSION IS A DEVASTATING DISEASE. IT AFFECTS NOT ONLY THE DIRECTLY afflicted but also the people around them, their families, and their closest relations. It indiscriminately hits all strata of society, no matter one's intellectual background, age group, or economic situation. There are many cases of highly successful and widely admired individuals who have been struggling with depression for years. Unfortunately, for reasons we still do not fully understand, this condition has been on the rise over the past decades. Considering its impact on an individual's quality of life and subsequently on the economy and society in general, gaining an understanding of what causes depression and trying to develop effective therapies is of utmost importance. Hence, this year's Neuroscience Special Issue is devoted to different aspects of depression.

Duman and Aghajanian (p. 68) review evidence from basic and clinical studies that stress and depression cause neuronal atrophy and decreased synaptic connections in cortical and limbic brain regions. Antidepressants can block or reverse these neuronal deficits, although typical antidepressants have limited efficacy; however, novel rapidly acting antidepressants produce a fast induction of synaptogenesis and quickly reverse synaptic deficits caused by chronic stress. Together, these findings support a synaptogenic hypothesis of depression and treatment response.

Newly generated neurons are required for mood control and for the beneficial effects of antidepressants. Understanding adult neurogenesis may therefore provide a road map for the development of new treatments for depression. Introducing the concept of the neurogenic interactome, Eisch and Petrik (p. 72) synthesize recent seminal findings relevant to neurogenesis, with special emphasis on the interchange between stress, depression, and cognition.

Developing a suitable animal model is often an important step for understanding the basic mechanisms underlying a disease. Berton *et al.* (p. 75) summarize the current models for mood disorders and attempt a prospective analysis of potential tests or tools that might be implemented to improve our repertoire of such models for depression. The problem of animal models is also touched on in an article on why mental illnesses are so hard to treat, part of a Mysteries of the Brain package in the News Focus section, starting on p. 30.

However, not all is bleak. There are individuals who overcome difficult situations and show astonishing resilience in the face of adverse circumstances and other forms of acute or chronic traumatic stress. Studying them might provide us with clues about what can go right. Southwick and Charney (p. 79) provide an overview of current ideas about why some people are more protected against stress and depression than others and how this knowledge may help us develop better treatments and successful prevention strategies.

In *Science Signaling*, Shah describes evidence that hyperpolarization-activated, cyclic nucleotide-gated channels may be therapeutic targets for the treatment of depression, and Anisman and Hayley describe how targeting inflammatory mediators may enhance the efficacy of conventional therapies for depression.

— PETER STERN

Depression

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Science

Synaptic Dysfunction in Depression: Potential Therapeutic Targets

Ronald S. Duman* and George K. Aghajanian

Basic and clinical studies demonstrate that depression is associated with reduced size of brain regions that regulate mood and cognition, including the prefrontal cortex and the hippocampus, and decreased neuronal synapses in these areas. Antidepressants can block or reverse these neuronal deficits, although typical antidepressants have limited efficacy and delayed response times of weeks to months. A notable recent discovery shows that ketamine, a *N*-methyl-D-aspartate receptor antagonist, produces rapid (within hours) antidepressant responses in patients who are resistant to typical antidepressants. Basic studies show that ketamine rapidly induces synaptogenesis and reverses the synaptic deficits caused by chronic stress. These findings highlight the central importance of homeostatic control of mood circuit connections and form the basis of a synaptogenic hypothesis of depression and treatment response.

Despite extensive research, the neurobiology of major depressive disorder (MDD) remains poorly understood due to lack of biomarkers, relatively low rates of heritability, and heterogeneity of precipitating factors, including stress (1–3). Studies of antidepressant mechanisms and the development of more effective therapeutic agents have also progressed slowly. The widely prescribed serotonin selective reuptake inhibitors (SSRIs), derived from drugs developed more than 50 years ago, take weeks to months to produce a therapeutic response and are only moderately effective, leaving more than one-third of depressed individuals resistant to drug treatments (3).

However, basic and clinical studies have begun to shed light on this widespread, debilitating illness that is characterized by loss of pleasure (anhedonia); decreased cognition and memory; and disrupted sleeping, eating, ambulation, and sexual activity. There are consistent reports of decreased size of brain regions implicated in depression, as well as neuronal atrophy, including loss of synapses in MDD and rodent chronic stress models. Recent studies report what is arguably the most important discovery in half a century: the therapeutic agent ketamine that produces rapid (within hours) antidepressant actions in treatment-resistant depressed patients (4, 5). Notably, the rapid antidepressant actions of ketamine are associated with fast induction of synaptogenesis in rodents and reversal of the atrophy caused by chronic stress (6, 7).

We propose a hypothesis that depression is caused by disruption of homeostatic mechanisms that control synaptic plasticity, resulting in destabilization and loss of synaptic connections in mood and emotion circuitry. We compare and contrast the mechanisms underlying typical anti-

depressants and ketamine, particularly the induction of synaptogenesis. Together, these studies provide a framework for current and future studies of the neurobiology of depression and for the treatment and prevention of MDD and other stress-related illnesses.

Neuronal Atrophy and Synaptic Loss in Depression

Brain-imaging studies of depressed patients provide strong and consistent evidence of decreased volume of cortical and limbic brain regions—such as the prefrontal cortex (PFC) and the hippocampus—that control emotion, mood, and cognition, suggestive of neuronal atrophy that is related to length of illness and time of treatment (8, 9). Functional imaging studies report reductions of connectivity of the hippocampus and the PFC, as well as other brain regions, although there are also reports of increased connectivity of some regions, indicating a more complex disruption of brain circuits (10, 11), possibly due to dysregulation of reciprocal connections (9). Accordingly, it has been proposed that dysregulation rather than an overall increase or decrease of connectivity within PFC networks and their target limbic regions are responsible for the disturbances in emotional, cognitive, and autonomic regulation in mood disorders (9). Also, whereas imaging provides connectivity information on a particular brain region, it does not provide information on the integrity and efficiency of connections, which will require further analysis.

Postmortem studies of MDD report a reduction in the size but not number of pyramidal neurons in the dorsal lateral (dl) PFC (12). There is also evidence that GABAergic interneurons (GABA, γ -aminobutyric acid) are decreased in the dlPFC, along with consistent reports of decreased glia (12, 13). Similar reductions in neuronal cell body size and neuropil have been reported in the hippocampus of patients with MDD (14). Magnetic resonance spectroscopy demonstrates

altered levels of GABA and glutamate cycling, indicating an imbalance of these major neurotransmitters in MDD patients (15).

Studies of neuronal morphology in human postmortem brain tissue have been hampered by poor fixation and tissue condition, but we recently reported that the number of synapses, determined by electron microscopy, is decreased in the dlPFC of depressed patients (16). Evidence in support of a reduction of synapse number is provided by reports of decreased levels of synaptic signaling proteins in MDD patients, including decreased levels of glutamate receptor subtypes, presynaptic neurotransmitter vesicle-associated proteins, and postsynaptic structural and functional proteins in the dlPFC, the hippocampus, and other forebrain structures (16–19). In many cases, the molecular alterations associated with depression can be distinguished from the medication status of the patients, although studies are not always sufficiently powered to make this distinction.

Stress Causes Neuronal Atrophy and Decreases Synaptic Density

Rodent studies have provided detailed evidence of neuronal atrophy, reduced synaptic density, and cell loss in models of depression and stress (Fig. 1). Chronic unpredictable stress (CUS), a putative model of depression, results in behavioral abnormalities, including core symptoms of depression such as anhedonia (7). CUS exposure causes a reduction in the length and branching of apical dendrites and decreases the number and function [i.e., reduction in serotonin [5-hydroxytryptamine (5-HT)] and hypocretin-induced excitatory postsynaptic potentials] of spine synapses in layer V pyramidal neurons of the medial PFC (Fig. 2) (7). Other chronic stress paradigms (such as restraint stress) cause similar reductions in dendrite complexity and spine density in PFC neurons and CA3 pyramidal cells of the hippocampus (20–22). Chronic stress also decreases neurogenesis (23) in the adult hippocampus and the number of glia in the medial PFC (2, 24), consistent with reports in postmortem MDD (12).

Atrophy of PFC pyramidal neurons is observed after as little as 1 week of restraint stress (20 to 30 min per day), indicating that these cells are particularly sensitive (20). High levels of social, environmental, and work-related stressors are experienced routinely in everyday life and probably cause PFC neuronal atrophy in many individuals. Dendrite complexity and synaptic density can also be increased or the effects of stress reversed by antidepressants, enriched environment, and exercise (6, 7, 21). In contrast, chronic stress causes hypotrophy of neurons in the amygdala and nucleus accumbens (24, 25), effects that could contribute to disrupted emotion, motivation, and reward behaviors that are regulated by these brain regions, demonstrating the involvement of a broader neurocircuit in the pathophysiology of depression.

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Mechanisms Underlying Disruption of Synaptic Homeostasis and Behavior

The reason that neuronal atrophy in response to stress has evolved is unclear. One possibility is that the mechanisms underlying these effects are designed to be engaged only briefly during the fight-or-flight response and then rapidly turned off when the stress and/or danger has subsided. Acute stress responses are necessary for shunting energy and resources to the appropriate organs and tissues for maximal responses to danger situations. However, continued or long-term stress exposure and sustained activation of the accompanying physiological signals have negative effects on brain and many other organ systems. This hypothesis is

supported by studies demonstrating that acute stress enhances glutamate transmission in the PFC and related cognitive function, but that chronic stress disrupts these processes (26).

One of the most frequently studied stress-activated systems is the hypothalamic-pituitary-adrenal (HPA) axis. Chronic exposure to adrenal glucocorticoids causes atrophy of neurons in the PFC and hippocampus, similar to the effects of chronic stress (20–22). Activation of the HPA axis and loss of negative feedback occur in more than 50% of depressed patients, highlighting the impact of this endocrine system in mood disorders.

Alterations of brain-derived neurotrophic factor (BDNF). Stress and adrenal-glucocorticoids in-

fluence neuronal systems at many levels, but neurotrophic factors (particularly BDNF) have been of particular interest with respect to atrophy of neuronal connections (2, 24). BDNF is required for neuronal development early in life and for neuronal survival and function, including activity-dependent synaptic plasticity, in the adult brain (24). Stress or glucocorticoid exposures decrease the expression of BDNF in the hippocampus and the PFC, and levels of BDNF are decreased in postmortem brains of depressed patients (2, 24). Conversely, chronic administration of a typical antidepressant increases BDNF expression in these brain regions, and the behavioral actions of antidepressants are blocked in BDNF deletion mutant mice (24). BDNF deletion mutants are also more vulnerable to stress (24, 27), and targeted deletion of BDNF in the hippocampus is sufficient to cause depressive behaviors (28). In contrast to the PFC and the hippocampus, BDNF activity in the mesolimbic dopamine system produces pro-depressive effects, demonstrating the region-specific actions of this plasticity-related factor (2).

As might be expected for an activity-dependent trophic factor, BDNF substantially affects dendrite complexity and spine density (Fig. 1). BDNF heterozygous deletion mutant mice exhibit decreased dendrite length and branching in the hippocampus and occlusion of the effects of stress (29, 30). Mice with a knock-in of a human loss-of-function BDNF gene variant (Val66Met) also show reduced dendrite length and decreased spine-synapse density, maturity, and function in the hippocampus and/or the PFC (Fig. 1) (27, 29, 31, 32). The Met polymorphism impairs the transport of BDNF mRNA to subsynaptic sites, a requirement for activity-dependent local translation of proteins essential for synaptic formation and maturation (Fig. 1) (27, 29, 31, 32). Brain-imaging studies demonstrate that carriers of the Met polymorphism have decreased hippocampal volume and reduced executive function (8, 29) and, if exposed to early-life stress, are more vulnerable to depressive symptoms (33).

Disrupted synaptic signaling pathways. Stress and depression also disrupt BDNF–tropomyosin-related kinase B (TrkB) receptor signaling, including reductions of the downstream extracellular signal-regulated kinase (ERK) and Akt pathways in the hippocampus and PFC (24, 34). These pathways positively influence synaptic maturation and stability via regulation of synaptic protein synthesis and glutamate receptor cycling (35, 36). Decreased Akt activity in the ventral tegmental dopamine neurons is associated with increased susceptibility to social defeat stress and is reversed by antidepressant treatment in rodents and in human postmortem tissue (37). In addition, stress and depression increase levels of a negative regulator of ERK signaling, mitogen-activated protein kinase phosphatase 1, that is sufficient to cause depressive behaviors (34) and decrease a downstream target of BDNF, neuritin, that is required for normal antidepressant behavior (38). Chronic

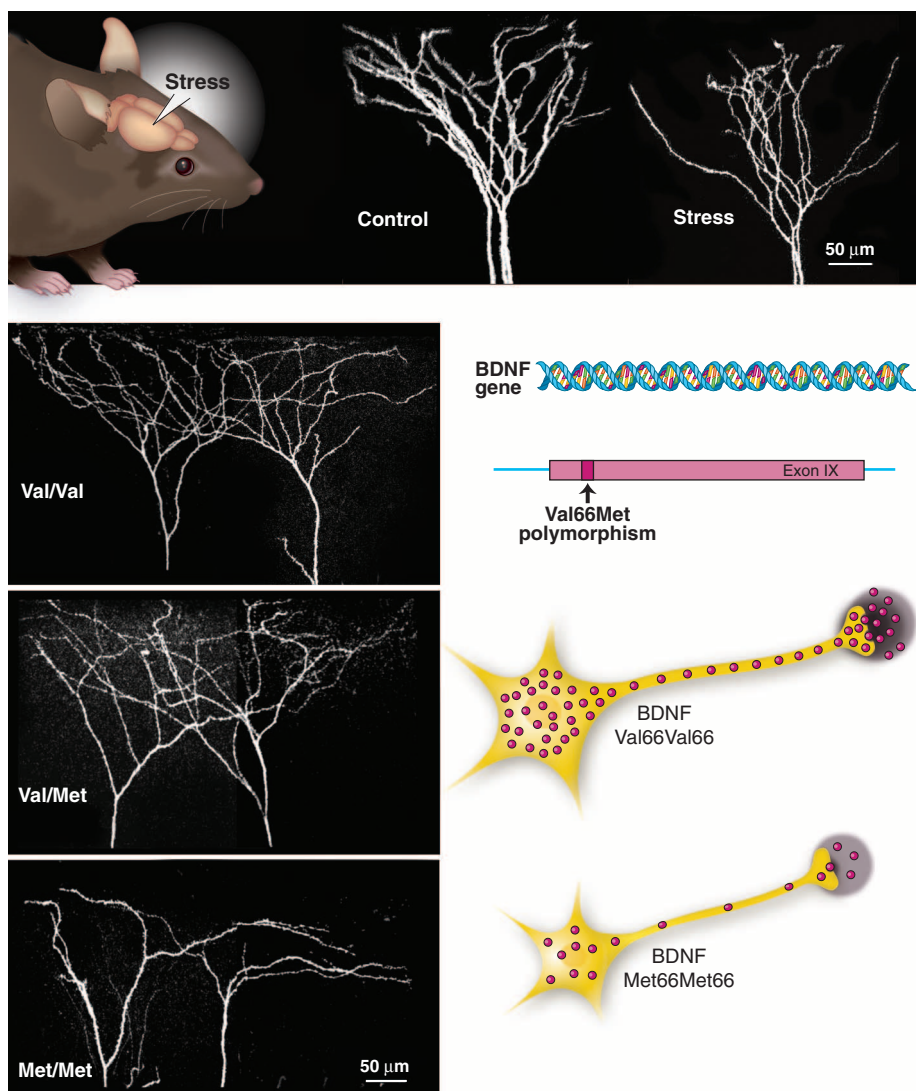


Fig. 1. Atrophy of cortical neurons is caused by chronic stress or a BDNF polymorphism. Representative confocal photomicrographs of labeled layer V pyramidal neurons in the medial PFC are shown. **(Top)** Effects of restraint stress (~30 min per day, 7 days) on dendrite length and branching. **(Bottom left)** Pyramidal neuron dendrites from wild-type (WT; Val66Val66) mice compared with mice with a knock-in of one or both Met alleles. **(Bottom right)** The Met allele decreases the transport of BDNF transcripts to dendrites and activity-dependent release of BDNF, which leads to atrophy of pyramidal neurons, including decreased number and length of dendrite branches. There is a gene dose effect, with Val66Met66 knock-in mice showing an intermediate effect and Met66Met66 mice a greater effect compared with WT Val66Val66 mice. Adapted from (20, 32).

Depression

stress also inhibits glutamate signaling and transmission via ubiquitin-mediated degradation of glutamate receptors in the PFC that are correlated with cognitive function (26). In addition, the reduction of synaptic density and proteins in response to stress and in the PFC of depressed patients is associated with increased expression of a transcriptional repressor of synaptic proteins (16).

Another signaling molecule that is regulated by BDNF-Akt signaling and is implicated in synaptic homeostasis is glycogen synthase kinase 3 (GSK3). GSK3 is involved in synaptic deconsolidation or pruning—the opposite of synaptogenesis—via regulation of glutamate receptor cycling (35). GSK3 is inhibited by Akt and is activated by protein phosphatase 1 (PP1). An excess of GSK3-deconsolidation could contribute to the reduction in spine synapses in response to stress and depression (Fig. 3) (39). Postmortem studies report increased levels of activated GSK3 in MDD or bipolar depression, in support of this hypothesis (39). Levels of GSK3 activity in the nucleus accumbens are also associated with social defeat stress, and blockade of GSK3 in this brain region or in response to systemic administration of a GSK3 inhibitor produces antidepressant effects in behavioral models of depression (39, 40).

Antidepressant Regulation of Synaptogenesis and Behavior

In contrast to the effects of stress and depression, typical antidepressants, with chronic treatment, produce a slow increase in neurotrophic factor expression and enhance synaptic plasticity. Moreover, the rapid-acting agent ketamine has fast and dramatic effects on synaptic function and spine density, providing further evidence for the relevance of synaptic alterations in depression and treatment response. Here, we provide a brief overview of typical antidepressants and synaptic plasticity and compare and contrast this with the actions of the rapidly acting drug ketamine.

Typical antidepressant drugs increase neuroplasticity. Evidence that typical antidepressants, such as the highly prescribed SSRIs, could influence synaptic plasticity is provided by electrophysiological studies demonstrating that chronic administration of fluoxetine enhances synaptic transmission and/or long-term potentiation (LTP), a cellular model of learning (41). Recent studies using elegant behavioral models of plasticity also provide evidence that chronic administration of fluoxetine reinstates ocular dominance plasticity in the adult mouse visual cortex and enhances the extinction of conditioned fear, an active learning process (42, 43). BDNF is required for the actions of fluoxetine on synaptic transmission, LTP, ocular dominance plasticity, and extinction training plasticity (41–43). There is also evidence that chronic antidepressant administration can increase spine density (44) or block the atrophy of dendrites and spines caused by chronic stress exposure (45, 46), although the role of BDNF in these effects has not been examined.

The behavioral response to antidepressants is blocked in BDNF deletion or BDNF Met knock-in mice (24, 27, 29).

Despite these positive neuroplasticity effects, SSRIs and other currently prescribed antidepressants have substantial limitations, including slow rates

of response and modest therapeutic efficacy. These limitations could be due to genetic or environmental factors that increase the severity of depression and/or resistance to treatment in certain individuals. However, these agents target only monoamines, which are modulatory transmitters and would not

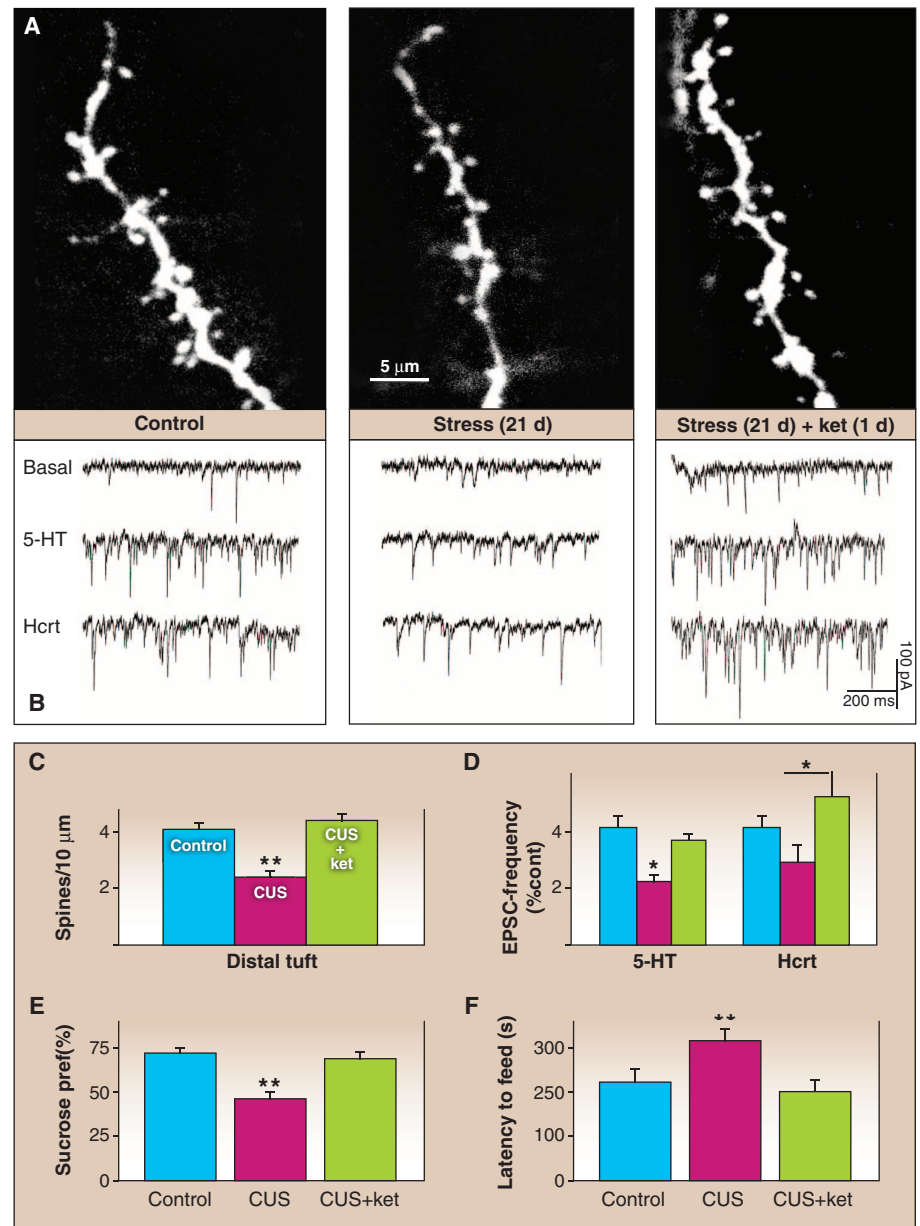


Fig. 2. Chronic stress decreases synaptic connections and produces depressive-like behavior: rapid reversal by ketamine. (A) Confocal photomicrographs of labeled layer V pyramidal neurons in the medial PFC, showing the effects of CUS (21 days) on spine-synapses in layer V pyramidal neurons and reversal by a single dose of ketamine 1 day later. (B) Effects of chronic stress ± ketamine administration on 5-HT- or hypocretin (Hcrt)-induced excitatory postsynaptic potentials (EPSPs). (C and D) Quantitative analysis of the effects of CUS ± ketamine on spine density and the corresponding regulation of spine synapse function, 5-HT- or Hcrt-induced EPSP frequency (percentage of control). (E and F) Influence of CUS ± ketamine on behavior in (E) the sucrose preference test (measured by percentage of preference for a sucrose solution) and (F) novelty suppressed feeding, measured by latency to feed in an open field (measured in seconds). These models provide measures of anhedonia and anxiety, respectively, and are rapidly (1 day) reversed by ketamine, compared with the requirement for long-term administration (3 weeks) of a typical antidepressant. Error bars indicate SEM; asterisks indicate significance from control (C to F) or between CUS and CUS+ket (E, Hcrt). Adapted from (7, 20).

be expected to produce a robust impact on synaptic transmission, activity-dependent release of BDNF, and synaptogenesis. Agents such as ketamine that produce more profound effects on fast excitatory glutamate transmission and increase BDNF release and synaptogenesis may be required to treat certain types or more severe forms of depression.

Rapid-Acting Antidepressants Increase Synaptogenesis

The recent discovery that ketamine, a *N*-methyl-D-aspartate (NMDA) receptor antagonist, produces rapid antidepressant responses in treatment-resistant depressed patients addresses the major limitations of available agents (4, 5, 47). A single dose of ketamine alleviates depressive symptoms within hours in patients who have failed to respond to two or more conventional antidepressants, and the effects are sustained for 7 to 10 days. Ketamine is also effective for bipolar depression and decreases suicide ideation (48). The fast, efficacious actions of ketamine by a different mechanism, NMDA receptor blockade, make this an exciting advance for depression therapeutics. The discovery that ketamine rapidly increases the number and function of synaptic connections has focused attention on synaptogenesis as a fundamental process for the treatment of depressive symptoms and also suggests that disruption of synaptogenesis and loss of connections underlies the pathophysiology of depression (Fig. 3).

Ketamine also produces fast antidepressant behavioral responses in rodent models, and these effects are correlated with rapid induction of synaptic proteins (within hours) and the number

and function of spine synapses in layer V pyramidal neurons of the PFC (6). Ketamine rapidly reverses the deficit in spine density, as well as the anhedonia and anxiety caused by exposure to chronic stress (3 weeks) (Fig. 2) (7). These effects are preceded by activation of the mammalian target of rapamycin (mTOR) signaling pathway and are blocked by infusions of the selective mTOR inhibitor rapamycin (Fig. 3) (6, 7). The mTOR pathway, which is localized to dendrites and cell bodies and regulates translation, has been linked to activity-dependent, long-term synaptogenesis reliant on protein synthesis (36).

Ketamine rapidly and transiently increases glutamate transmission in the PFC, possibly via inhibition of tonically active GABAergic interneurons, and the resulting burst of glutamate is thought to underlie ketamine induction of synaptogenesis (6, 7). In addition, the synaptogenic actions of ketamine are blocked in mice with a knock-in of the BDNF Met allele that impairs BDNF mRNA transport to dendrites, as well as in conditional BDNF mutant mice (32, 49). These findings are consistent with cell culture studies demonstrating that glutamate receptor stimulation of synaptogenesis requires the release of BDNF to stimulate TrkB-mTOR signaling and synaptic protein synthesis (50). Activity-dependent release of BDNF could also explain why ketamine is more rapid and efficacious than conventional antidepressants, which increase the expression but not the release of BDNF. A requirement for the synthesis of BDNF, as well as other synaptic proteins, is supported by evidence that inhibition of elon-

gation factor 2 kinase and desuppression of translation are required for the actions of ketamine (49).

Ketamine also rapidly increases activity-regulated cytoskeleton-associated protein (Arc) (6), which is required for actin polymerization and stable expansion of dendritic spines (Fig. 3) (51). The actions of ketamine also require inhibition of GSK3 (52). As discussed above, GSK3 could contribute to synaptic deconsolidation and is increased in brains of MDD patients (39). The actions of ketamine may include blockade of NMDA receptors that lead to activation of GSK3 via PP1, thereby causing spine destabilization (35).

The rapid antidepressant actions of ketamine could involve a number of cortical and limbic brain regions, many of which are reported to show alterations of glutamate transmission and synaptic activity. We have found that infusion of rapamycin into the medial PFC, but not the dorsal striatum, blocks the effects of systemic ketamine administration (6). Additional blocking studies, as well as local infusions of ketamine, are needed to further define the brain regions and circuits underlying the rapid actions of ketamine. Also, because the interpretation of preclinical studies is limited by the animal models available, additional human-imaging studies will be needed to confirm the circuitry underlying the clinical response to ketamine.

New rapid-acting therapeutic targets. The psychotomimetic effects and abuse potential limit the development and use of ketamine for the treatment of depression. However, characterization of the mechanisms underlying the actions of ketamine has led to new potential targets for drug development.

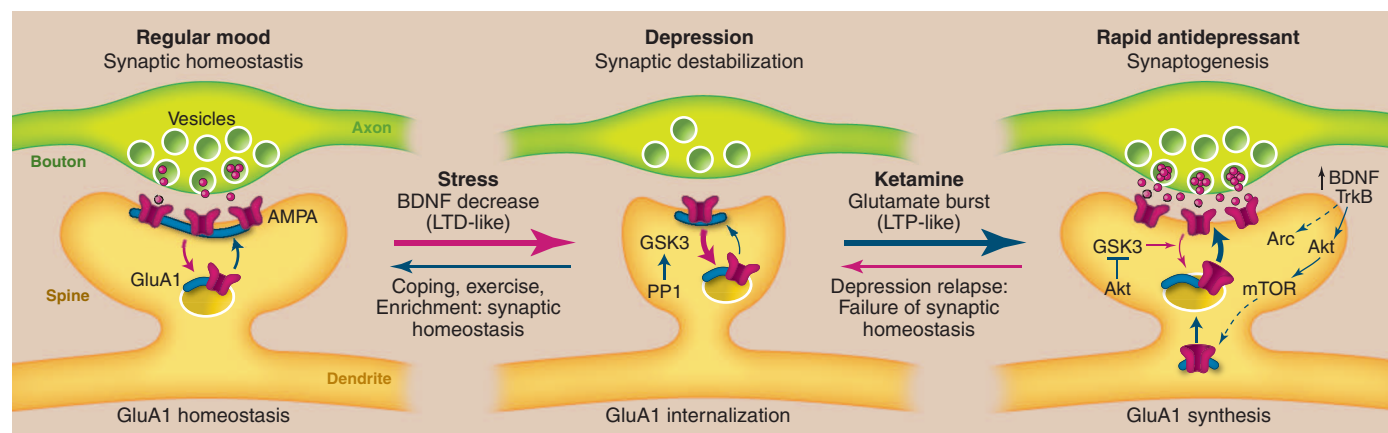


Fig. 3. Model depicting the synaptogenic basis of depression and treatment response. **(Left)** Regular mood (synaptic homeostasis). Under normal conditions, levels of synapse number and function are maintained by homeostatic mechanisms that contribute to regular mood. This includes cycling of glutamate A1 (GluA1) receptors to and from the synapse. **(Middle)** Depression (synaptic destabilization). Chronic stress exposure decreases synaptic density, similar to the destabilization of spine synapses that occurs under conditions that cause long-term depression (LTD). Neuronal atrophy and synaptic loss are reversible, possibly more rapidly in resilient individuals with coping, exercise, or enriched environment. Stress also decreases BDNF, and BDNF deletion mutant mice exhibit similar synaptic deficits, suggesting that this neurotrophic factor may contribute to the loss of synapses and neuronal atrophy. GSK3,

which is increased in depression, can be activated by PP1 and may also contribute to synaptic destabilization by promoting the internalization of GluA1. **(Right)** Rapid antidepressants (synaptogenesis). Ketamine rapidly increases glutamate transmission and synaptogenesis, similar to LTP. Ketamine-induction of synaptogenesis requires BDNF/TrkB-activation of Akt and mTOR signaling, resulting in increased translation of synaptic proteins, including GluA1, as well as Arc, which is required for the expansion and stabilization of spines. The actions of ketamine are also dependent on inhibition of GSK3, which could occur via stimulation of Akt or by blockade of NMDA receptors and PP1 (not shown in the figure). Depressed patients treated with ketamine relapse after 7 to 10 days, possibly due to failure of synaptic homeostasis, which could result from genetic mutations or environmental factors such as sustained stress.

Basic and clinical studies show that selective NMDA receptor subtype 2B (NR2B) antagonists produce antidepressant behavioral and synaptogenic actions (6, 24). However, the clinical response to NR2B selective agents does not appear to be as rapid as the response to ketamine, and further studies are needed to address this issue, as well as the efficacy of these agents in treatment-resistant patients. Because the actions of ketamine require a burst of glutamate transmission, agents that either increase synaptic levels of glutamate or act at postsynaptic sites to enhance glutamate receptor signaling have been investigated. Blockade of mGluR2/3 receptors, which are located presynaptically and regulate the release of glutamate, produces rapid behavioral responses that require mTOR signaling (53, 54). Positive AMPA receptor potentiating agents produce antidepressant behavioral responses in rodents and stimulate mTOR signaling in cultured cells (15, 50), but further studies are required to determine if the behavioral effects are dependent on mTOR signaling and synaptogenesis.

Inhibition of GSK3, which is required for the actions of ketamine in rodent models (52), could also produce rapid ketamine-like effects or enhance the response to ketamine. Other neurotransmitter systems that indirectly increase glutamate via regulation of tonic firing of GABAergic interneurons may also produce ketamine-like effects. For example, the roles of glutamate, synaptogenesis, and mTOR signaling in the rapid antidepressant actions of scopolamine, a muscarinic receptor antagonist (55), are currently under investigation.

Conclusions

Evidence exists for a central role of homeostatic control of synaptic connections in key mood-related

circuits in the etiology and treatment of depression. Further studies are required to determine the effects of stress and antidepressants on synaptogenesis in other circuits and populations of neurons (e.g., anterior cingulate cortex, nucleus accumbens, amygdala) and to uncover the functional importance of synaptic alterations on behavior in models of depression and anxiety, as well as cognition, learning, and memory. These studies will further define the neuronal and synaptic alterations underlying depression and will contribute to the development of safer, more efficacious antidepressants that last longer and act faster.

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PERSPECTIVE

Depression and Hippocampal Neurogenesis: A Road to Remission?

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Adult-generated hippocampal neurons are required for mood control and antidepressant efficacy, raising hopes that someday we can harness the power of new neurons to treat mood disorders such as depression. However, conflicting findings from preclinical research—involving stress, depression, and neurogenesis—highlight the complexity of considering neurogenesis as a road to remission from depression. To reconcile differences in the literature, we introduce the “neurogenic interactome,” a platform from which to consider the diverse and dynamic factors regulating neurogenesis. We propose consideration of the varying perspectives—system, region, and local regulation of neurogenesis—offered by the interactome and exchange of ideas between the fields of learning and memory and mood disorder research to clarify the role of neurogenesis in the etiology and treatment of depression.

Major depressive disorder is a leading cause of disability worldwide (1). There is great need for improved understanding of both the pathophysiology of depression and the neuromechanisms of antidepressant ther-

apeutics. One relatively novel but prevalent area of research focuses on the neurogenic hypothesis of depression. As one index of its relevance, PubMed citations related to this hypothesis (search terms “neurogenesis” and “depression”)

increased 14-fold from when it was first introduced (1999 to 2001, 21 citations) to 2011 (2009 to 2011, 309 citations). How did this hypothesis gain traction so quickly? And more importantly, after a decade of research, does the neurogenesis hypothesis of depression still hold promise for paving a road to remission from depression?

In its simplest form, the neurogenic hypothesis of depression states that new neurons in the adult brain are needed for proper mood control and for antidepressant efficacy (2). Once a controversial phenomenon, the generation of new neurons in discrete regions of the adult brain of most mammals, including humans, has been repeatedly confirmed by using modern techniques (3). One of these neurogenic niches, the subgranular zone of the dentate gyrus (Fig. 1, A to C), lies within the hippocampus. Because the hippocam-

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pus is a brain region involved in memory and mood control, the discovery of adult neurogenesis launched two parallel investigations into its functional roles in memory and mood control (2, 3). The neurogenic hypothesis of depression quickly became popular because of a wealth of correlative studies. Humans with depression had decreased hippocampal volume (4, 5); decreased neurogenesis could lead to a smaller hippocampus. In laboratory animals, antidepressants enhanced hippocampal neurogenesis and did so with a lag time similar to the delay between antidepressant administration and clinical efficacy in humans (6), reflecting the time of neuronal maturation in the adult hippocampus (3). In nonhuman primates, stress (a predisposing factor to depression in humans) decreased neurogenesis, and neurogenesis levels were normalized by antidepressants (7). These and many other studies gave the neurogenic hypothesis of depression its initial robust trajectory (2): Adult-generated hippocampal neurons are needed for proper mood control (Fig. 1D) and for antidepressant efficacy (Fig. 1E). Eventually, such studies paved the way for causative research showing that ablation of hippocampal neurogenesis in mice indeed impairs antidepressant efficacy (8).

The neurogenic hypothesis of depression jump-started the field in regard to publications and ideas, but conflicting results have raised red flags about its validity. In laboratory animals, ablation of neurogenesis does not always cause depressive-like symptoms (9), stress does not always decrease neurogenesis (10), and some effects of antidepressants are neurogenesis-independent (11). Clinically, people with mood disorders have hippocampal dysfunction and altered stress response, but these might be predisposing rather than causative factors in depression (1, 4). Also, neurogenesis clearly persists in the adult human hippocampus (12, 13), but the levels are so low relative to laboratory animals that questions have been raised about whether alterations in neurogenesis could result in a functional change (either positive or negative) in affect or memory.

Neurogenic Interactome

Do these findings spell “the end of the road” for the neurogenic hypothesis of depression? From our perspective, they do not. We propose that differences in the literature can be accounted for by considering the diverse and dynamic factors regulating neurogenesis, using a model we refer to as “neurogenic interactome” (Fig. 1F). The neurogenic interactome consists of key endocrine and neurochemical signaling cascades, reciprocal connections among brain regions that control adult neurogenesis, and their major downstream influences on behavior. Below we briefly show how evaluation of three elements in our model—interplay of different brain structures, the diverse functions of the hippocampus, and the heterogeneous components of the neurogenic niche—

reconciles apparent discrepancies in the neurogenic hypothesis of depression and lays a roadmap for future research.

First, the neurogenic interactome highlights the importance of input connections to hippocampus in regulation of adult neurogenesis. Anatomical connections among the hippocampus, dentate gyrus, and brain regions important for stress and fear and emotional memory (4) help to explain the involvement of adult-generated neurons in stress and depression (Fig. 1B,F). The hypothalamic-pituitary-adrenal (HPA) axis and stress hormones have long been known to influence adult neurogenesis (2). Recently, it was shown that adult neurogenesis reciprocally regulates the HPA axis, buffering the stress response (14). This ability of new neurons to put the brakes on the stress response appears important in times of moderate to extreme or unpredictable stress (14–16), but not as important under normal conditions or mild or predictable stress (9), and may be imposed by direct regulation of the hypothalamus. Although this connection is yet to be experimentally tested, it is known that inactivation of the amygdala, a limbic system involved in emotional memory, suppresses hippocampal neurogenesis (17). Thus, stimulation of the amygdala under specific conditions [such as predictable mild stress (18)] may directly up-regulate adult neurogenesis.

Consideration of the involvement of stress and the connections in the neurogenic interactome may also help to explain the apparent impression from the literature that certain behavioral tests are neurogenesis-dependent, whereas others are not, and why many effects of antidepressants are neurogenesis-independent (2, 11). Different behavioral tests for depression and anxiety induce different levels of stress and/or fear and thus activate different subsystems of the neurogenic interactome (Fig. 1F). This secondarily can modulate the behavioral output of these subsystems (1) and also influence adult neurogenesis and neurogenesis-dependent behavior (Fig. 1F). Lastly, although clearly unpredictable stress and stimulation of the stress axis decrease neurogenesis and can lead to depression (2), these connections explain why it is not inevitable that “stress decreases neurogenesis” (9, 14–16, 18). Rather, coping with stressful events requires neurogenesis, and predictable stress may be beneficial for both neurogenesis and mood control (16, 18). The learned ability to inhibit fear and to cope with stress serves as a behavioral antidepressant, which correlates with up-regulated neurogenesis (16, 19). These preclinical findings map well with studies in human as well as nonhuman primates (12, 15, 16). Interestingly, there are similarities between endogenous stress-coping strategies and the neuromechanism of antidepressants (20, 21). This suggests that antidepressants may exploit the same neural substrates as behavioral modifications. Mechanistically, the unique contribution of adult-generated neurons to hippocampal function during psycho-

social stress or “when the road gets rough” is in part due to the unique physiological characteristics and anatomical connections of these neurons (3).

Mood and Memory

Second, the neurogenic interactome underscores that the control of hippocampal neurogenesis over mood and memory may be more interrelated than previously appreciated. Adult-generated hippocampal neurons provide a type of neuronal plasticity, pattern separation, that allows acquisition and separation of closely spaced memories (22, 23). This is in contrast with embryonic-generated hippocampal neurons that are necessary for pattern completion and recall of memories (23). There is a clear connection between adult neurogenesis, pattern separation, and learning and memory; for example, diminished neurogenesis decreases pattern separation and learning/memory, whereas enhanced neurogenesis improves them (2, 22, 23). However, it is unclear how adult neurogenesis controls pattern separation and concurrently influences mood, and vice versa (Fig. 1F). Pattern separation may not only be important for learning and memory but also for recognizing dangerous and stressful signals, such as cue recognition during conflict or stress, thus triggering or aggravating anhedonic or depressive behavior (24). Interestingly, mice with stress-induced social avoidance have increased adult neurogenesis (10), suggesting they may have a better ability to discern or remember harmful signals. Prevention of this increased neurogenesis diminishes social avoidance (10), which can be interpreted as inability to cope with a stressor. Pattern separation deserves evaluation not only for its involvement in spatial learning and memory but also in the development or persistence of mood disorders, because recognizing stressors or danger shares similar substrates with learning and memory (Fig. 1F). Because strong and unpredictable stress decreases adult neurogenesis, the cognitive functions dependent on pattern separation will be impaired, only worsening the ability to recognize and cope with further incoming stressful events and situation, thus creating a vicious cycle.

Neurogenic niche

Third, although not depicted in this interactome schematic (Fig. 1F), the heterogeneous components of the neurogenic niche play key roles in determining the ultimate influence of adult neurogenesis on behavior and response to antidepressants. Adult-generated neurons are critical for dentate gyrus-related behavioral output and the neurobehavioral effects of antidepressants (2, 3). Despite their small number, they have a disproportionate influence on hippocampal circuitry and behavior (3), underscoring that “the dentate gyrus is not a democracy”. However, other components of the niche—such as vasculature, astrocytes, and microglia (3)—regulate neurogenesis and respond to antidepressants, even in

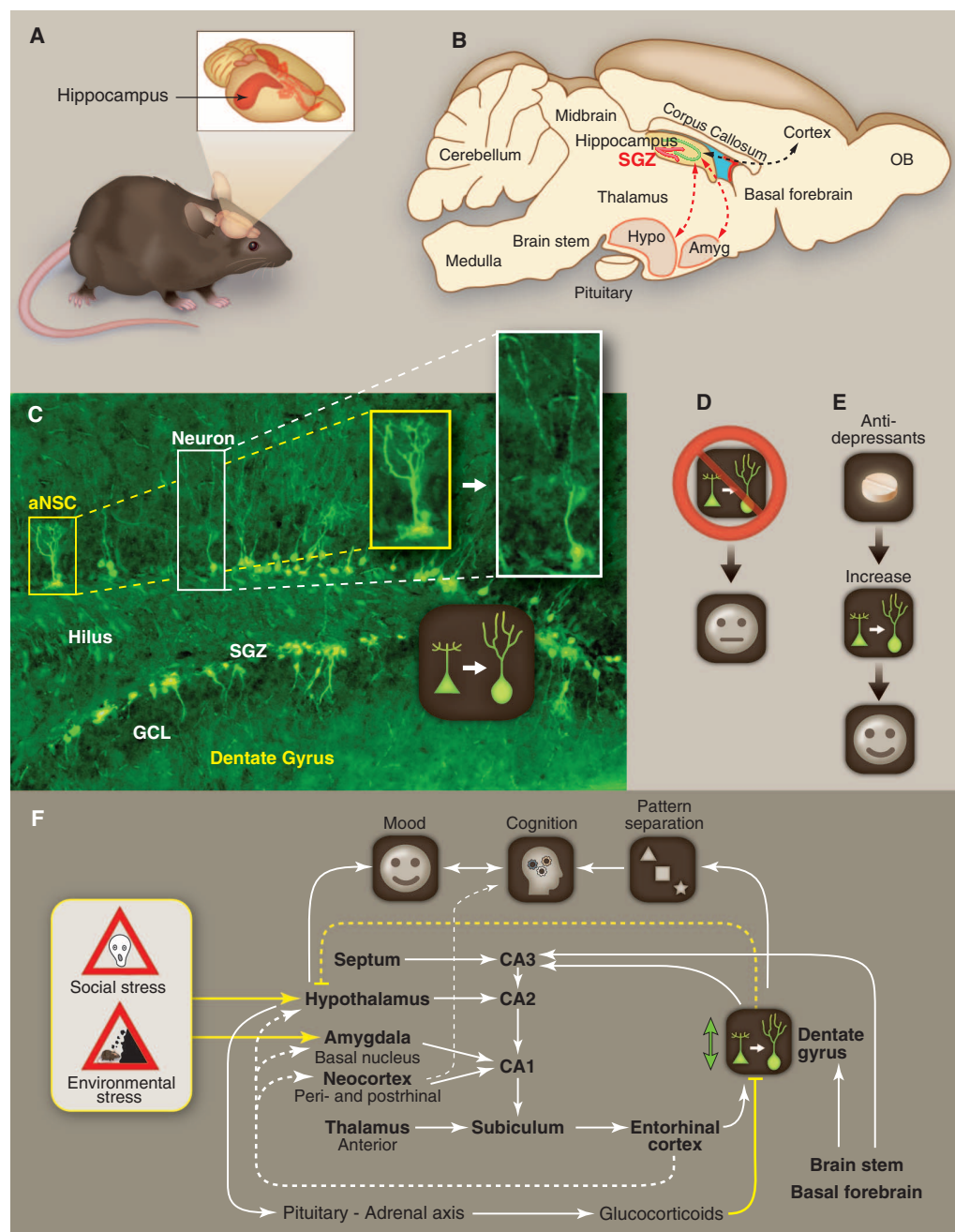


Fig. 1. Adult hippocampal neurogenesis in depression. **(A)** Neurogenic niches in the mouse brain (inset, red) generate neurons throughout life. **(B)** The hippocampus interacts with other major brain regions (e.g., hypothalamus, Hypo; amygdala, Amyg; dotted lines) to contribute to brain functions like memory and mood. The hippocampal dentate gyrus hosts one neurogenic niche, the subgranular zone (SGZ, red). OB, olfactory bulb **(C)** Photomicrograph of the SGZ in a transgenic mouse depicts the process of neurogenesis, beginning with an adult neural stem cell (aNSC, yellow frame) and ending with a dentate gyrus neuron (white frame) that fully integrates into the granule cell layer (GCL). Neurogenesis is a process, not a time point (2), a message emphasized in the road sign (inset) that condenses the journey from aNSC to GCL neuron. **(D and E)** The two branches of the neurogenic hypothesis of depression. **(D)** The first branch proposes ablation of adult neurogenesis does not greatly influence mood under normal, nonstressful conditions. **(E)** The second branch proposes antidepressants may confer their effects on improving mood via up-regulating adult neurogenesis. **(F)** The neurogenic interactome. Both direct

and indirect anatomical connections (solid and dashed white lines, respectively) influence adult neurogenesis in a dynamic manner (double-headed green line). Alterations in neurogenesis reciprocally influence the connecting regions (dashed white line). For example, intact neurogenesis inhibits the hypothalamus (dashed yellow line) and the “stress axis,” including the HPA axis, thus contributing to mood control. In contrast, during stress, the HPA axis and limbic system are activated (solid yellow lines). This influences both levels of neurogenesis and the reliance of key brain functions, like cognition and mood, on adult-generated hippocampal neurons. Thus, the stress experience (length, duration, type) influences the importance of adult-generated neurons in buffering the stress response. Moreover, involvement of adult-generated neurons in pattern separation and thus cognition can protect against depression by improving recognition and coping with stressors. The two major functions of adult-generated hippocampal neurons, mood/stress control and learning/cognition, help explain the involvement of adult neurogenesis in etiology of depression-like phenotype and guide design of novel antidepressants.

the human hippocampus (12). Aside from niche components, stem and progenitor cells should also be considered for their contribution to neurogenic control and mood regulation. They do not make or receive traditional neuronal synapses or connections and have previously been considered passive participants in the neurogenic niche. However, we now know that stem and progenitor cells provide structural, biochemical, and metabolic signals to the niche that can regulate neurogenesis (3). Thus, we have to investigate how each individual component of the niche is influenced by potential antidepressants and consider components of the niche themselves as specific targets for novel antidepressant therapeutics.

The neurogenic hypothesis of mood disorders remains promising for conceptualizing depression mechanisms, which may lead to novel avenues for treatments. However, more work needs to be done. The typically parallel lines of research on memory and mood should be merged to evaluate, for example, whether enhanced pattern separation enhances stress coping and levels of neurogenesis in laboratory animals (19, 23). Other preclinical experiments are needed to fine-tune the neurogenic interactome, assessing whether stimulation of the amygdala enhances neurogenesis and whether other brain regions involved in depression, like the nucleus accumbens, are also influenced by levels of neurogenesis. Clin-

ically, we need more information on the level and regulation of human neurogenesis, particularly in the brains of depressed humans (12). Interestingly, neurogenesis decreases with age in humans and animals (13, 25), whereas depression prevalence increases with age. More research is warranted to examine to what extent the age-induced increase in depression is due to life experience, age-induced increase in medical burden, or possibly age-induced decrease in neurogenesis (4, 5, 12, 25). Also, in vivo imaging of correlates of human neurogenesis is feasible (25, 26), but greater technical advances are needed before we can conclude what aspects of neurogenesis structure and function are shared between humans and laboratory animals. Although standard assessment of human neurogenesis in vivo is many years away, a destination on the road to remission for people with mood disorders may eventually be customized diagnostic evaluation that takes into account their individual levels of adult neurogenesis.

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PERSPECTIVE

Are We Getting Closer to Valid Translational Models for Major Depression?

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Advances in characterizing the neuropathology and functional dysconnectivity of depression and promising trials with emerging circuit-targeted and fast-onset therapeutics are providing unprecedented opportunities to gain deeper insight into the neurobiology of this devastating and pervasive disorder. Because of practical and ethical limitations to dissecting these mechanisms in humans, continued progress will critically depend on our ability to emulate aspects of depressive symptomatology and treatment response in nonhuman organisms. Although various experimental models are currently available, they often draw skepticism from both clinicians and basic research scientists. We review recent progress and highlight some of the best leads to diversify and improve discovery end points for preclinical depression research.

Models of psychiatric illness rely on manipulation of known etiological or risk factors to induce tractable behavioral or physiological symptoms in animals. To be considered valid for hypothesis testing and therapeutic development, modeled symptoms are expected to show a reasonable homology with those of human disease and should respond to clinically effective treatments, with clinically relevant time courses. Modeling major depressive disorder

(MDD) along these lines poses a number of substantial challenges.

Lost in Translation: Why Animal Models Struggle with Major Depression

A first obstacle is our limited understanding of the multifactorial pathogenic processes that underlie affective, cognitive, and homeostatic abnormalities in MDD. Given the subjective nature of most core symptoms and the lack of valid

biomarkers, establishing links between clinical variables and animal end points cannot currently be done without a certain degree of interpretation or anthropomorphism.

Further complication derives from the extreme heterogeneity of the illness and from the difficult delineation of its boundaries with health and other psychiatric conditions (1). A good example is the symptomatic facet of anhedonia (or loss of pleasure and interest) that is considered a cardinal feature of MDD but is truly present only in up to 50% of MDD patients and is very prominent in other conditions, such as schizophrenia (2). Our growing appreciation of individual patterns of functional disconnections and genetic variability associated with MDD suggests that the current diagnostic approach collapses a myriad of distinct pathophysiological states that are unlikely to be captured comprehensively by any single experimental model (3).

Not surprisingly, given this clinical and etiological heterogeneity, the therapeutic effects of current antidepressants, which all share monoaminergic neurotransmission as a single common

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target, are often inconsistent (about 50% of patients will not respond to a first-line treatment and placebo-expectancy effects are typically larger than specific drug effects) (1). This therapeutic inconsistency, too, has important implications for modeling, as it impedes the use of reference drugs in animals as a clear-cut criterion for validation or invalidation.

Despite these problems, we have many heuristic behavioral paradigms for the study of MDD, and several have proven undeniably useful for the rational development of monoaminergic antidepressants. These models generally capitalize on the epidemiological evidence that stress and adverse psychosocial experiences (such as interpersonal violence, neglect, or unwanted separation) often precede the onset, or predict the recurrence, of depressive episodes. Most of these paradigms were developed 30 years ago and have been applied since with little modifications. Given the failure of virtually all clinical development programs with candidate therapeutics based on non-monoaminergic mechanisms during the past decades, the capability of current models to detect antidepressants with truly novel mechanisms has been repeatedly questioned. Several comprehensive reviews have been published in recent years discussing the pros and cons of each model and proposing guidelines for their improvement, with a strong consensus on the need to better incorporate etiological factors in the design of novel paradigms (4, 5).

During the past 5 years, we have witnessed a number of key conceptual and technical advances that hold the promise of transforming the landscape of preclinical MDD research. We highlight some of these recent developments and attempt a prospective analysis of how they may contribute to improve and diversify discovery end points for depression. We focus on two areas of need: (i) the need to translate our improved understanding of depression circuitry into behavioral models of MDD and (ii) the need to translate improved knowledge of risk genes and molecular neuropathology into models of MDD.

Toward Models of Circuit-Centered Symptomatic Dimensions in MDD

To address the problem of MDD's clinical heterogeneity, an approach that has emerged over the last decade is the recasting of MDD symptomatology into dimensional intermediate phenotypes defined using quantifiable and objective behavioral or neural end points (3). Examples of such end points relevant to the spectrum of MDD symptoms include measures of whole-brain connectivity under resting state or in response to hedonic or aversive stimuli, as well as standardized behavioral measures of hedonic capacity, emotional reactivity, and nonverbal social attunement. Although integration of these neurobehavioral variables seems to afford a discriminative power for pathology approaching that of psychometric

constructs (2, 6–9), large-scale trials necessary to establish their potential as diagnostic tools are still lacking. Nevertheless, efforts to validate and diversify this type of translatable variable, proximal to circuit function, are critical to bridge the divide between MDD clinical phenomena and preclinical models. A complementary aspect of this effort, on the preclinical side, consists in dissecting circuit modules that underpin aligned phenotypic domains in animals. Emergent techniques allowing temporally precise genetic control of circuit activity (10) and real-time monitoring of interregional connectivity (11) in behaving animals as well as dynamic imaging of local circuits (12, 13) are facilitating this task. We highlight below a number of recent studies using these approaches to explore behavioral domains related to negative affect, positive affect, socioaffective functioning, and cognitive function.

Negative affect. The behavioral end point related to “negative affect” that the majority of rodent studies examines using simple behavioral tasks (like the forced swimming test) or more elaborate paradigms (like the learned helplessness test) is a form of behavioral passivity and quiescence that develops in many species upon exposure to uncontrollable stress. Stress-induced escape deficits in these so-called “despair” tests can be delayed or normalized by antidepressants. Although this class of test is often labeled as being selectively predictive of the clinical activity of monoaminergic antidepressants, recent studies with the *N*-methyl-D-aspartate antagonist ketamine (14) or deep brain stimulation (DBS) of the infralimbic cortex (the rodent homolog of the cg25 area) (15) have revealed robust antidepressant-like responses that cannot be explained through direct monoaminergic mechanisms. On the basis of these data, it may seem necessary to update certain of our views about these paradigms and to improve our understanding of underlying circuitry.

Using novel methods for high-resolution tracking of motor responses in freely moving rats and simultaneous recording of medial prefrontal cortex (mPFC) neurons, Warden *et al.* (16) were able to identify a cluster of cells that predict active escape attempts in the forced swimming test. By optogenetically controlling the activity of these neurons, they demonstrated their causal role in the animal's “decisions” to struggle or stay passive in the test. Not all pyramidal neurons in the mPFC contributed to the initiation of goal-directed escape behaviors but only a specific distributed subset defined by their axonal connections with the raphe nuclei, the region in the brainstem that contains the bulk of serotonin neurons. These results converge with those of several other groups to suggest that, as drug screens, the so-called “despair” tests may specifically probe mechanisms by which the prefrontal cortex exerts top-down control over ancestral circuits implicated in the generation of aversive states (such as panic and dysphoria) and comprising serotonergic neurons.

These results reinforce the construct validity of this class of tests and may facilitate the development of reverse-translational tasks to interrogate parallel antidepressant-regulated circuits in humans.

The lateral habenula (LHb) is another brain region heavily implicated in the descending control of monoaminergic circuits. It has also recently been implicated in the generation of aversion and antidepressant-sensitive escape deficits in the learned helplessness test (17, 18). Here again, virally mediated track tracing and optogenetics were used to identify specific neuronal subpopulations in the LHb that affect behavior and to pinpoint their downstream projections to the ventral tegmentum as one critical output. The circuitry of the habenula is highly conserved, and its role in the encoding of negative affective states like “disappointment” or “fear” extends from the zebrafish to primates. DBS of the LHb is considered a possible therapeutic approach for MDD after a number of promising case reports.

Positive affect. Reductions in “positive affect” and hedonic capacity are commonly observed in MDD and contribute to the complex construct of anhedonia (19). Evidence for fast antianhedonic effects of DBS in the nucleus accumbens in treatment-resistant MDD patients implicates this highly conserved component of the mesolimbic reward pathways, frequently studied for its role in motivation and addictive processes. A number of recent studies using genetic approaches to trace, identify, and silence neurons have started providing new insights into neurophysiological mechanisms through which chronic stress in rodents represses preference for highly palatable foods, an anhedonia-like symptom reversed by chronic antidepressants. By implicating synaptic changes affecting specific neuronal subtypes in this heterogeneous brain region, such as dopamine medium spiny neurons expressing the dopamine D1 receptor (20) or cholinergic interneurons (21), these studies have started tracing a wiring diagram that may prove instrumental in refining electrode-based strategies for treatment of anhedonia.

Socioaffective function. Deficits of interpersonal functioning are another important component of MDD symptomatology. These deficits have been linked to endophenotypes such as deficient nonverbal attunement, alterations in the processing of socioaffective information, and selective serotonin reuptake inhibitor (SSRI)-sensitive reductions in socially evoked patterns of cortic limbic activity (22). Studies in apes, monkeys, and other smaller primates (such as the tree shrews) have provided the best evidence for the occurrence of stress-induced “pathological” deviations of social behaviors in animals, paralleling aspects of human MDD symptoms. Two of the most salient aspects of socioaffective alterations in nonhuman primates exposed to “ethological” stressors (such as periods of isolation during early development or forced subordination in adulthood) include expression of a stereotypical prostrated and socially

unresponsive posture (Fig. 1) and the exacerbation of socially submissive behaviors. These symptoms, reported both in captive and free-ranging primates (23), appear partly reversible by administering chronic SSRIs and co-occur with cardiovascular, neuroendocrine, and neural abnormalities common in clinical depression, such as decreased hippocampal volume (24) and decreased neurogenesis (25). Although ethologically valid primate models of socioaffective deficits have a strong face-validity with MDD, our capability to investigate their underlying circuitry is limited by technical and ethical considerations.

Rodent social stress models such as the social defeat paradigm (26) or tests of social dominance such as the “tube test” of social competition (27) provide a number of valid alternatives to examine neurobiological underpinnings of socioaffective behaviors. The relatively long-lived and

antidepressant-sensitive form of social avoidance that develops in a subset of mice subjected to repeated experience of aggression (in conjunction with an array of other motivational and metabolic changes) has provided a reliable end point to examine differential circuit adaptations associated with social aversion or resilience to social stress (26). Electrophysiological studies in this model point to alterations in several subcortical circuits (partly overlapping with those involved in anhedonia (28, 29) and negative affect (30) highlighted above) that discriminate between socially avoidant animals and their resilient counterparts. In vivo multiregion electrophysiological techniques have also revealed altered patterns of corticolimbic synchrony in defeated mice (31), which suggests that stress-induced alterations in the connections between the mPFC and downstream targets in the limbic system (such as the

amygdala) may be implicated in the development of socioaffective phenotypes relevant to MDD. This hypothesis is in line with the recent demonstration that high-frequency photostimulation of pyramidal neurons in the mPFC of defeated mice or genetic manipulations affecting synaptic efficacy in the same circuits induce antidepressant-like responses in tests of social aversion (32) and social competition (33).

Cognition. Cellular and molecular correlates of cognitive deficits in MDD (such as deficits in attention and executive function) are understudied. There is a great need for novel nonhuman behavioral models to address these mechanisms, and it is good news that a number of such paradigms are starting to emerge, in primates (indispensable to approach mechanisms underlying higher cognition) as well as in rodents. A core concept of cognitive theories of depression is the

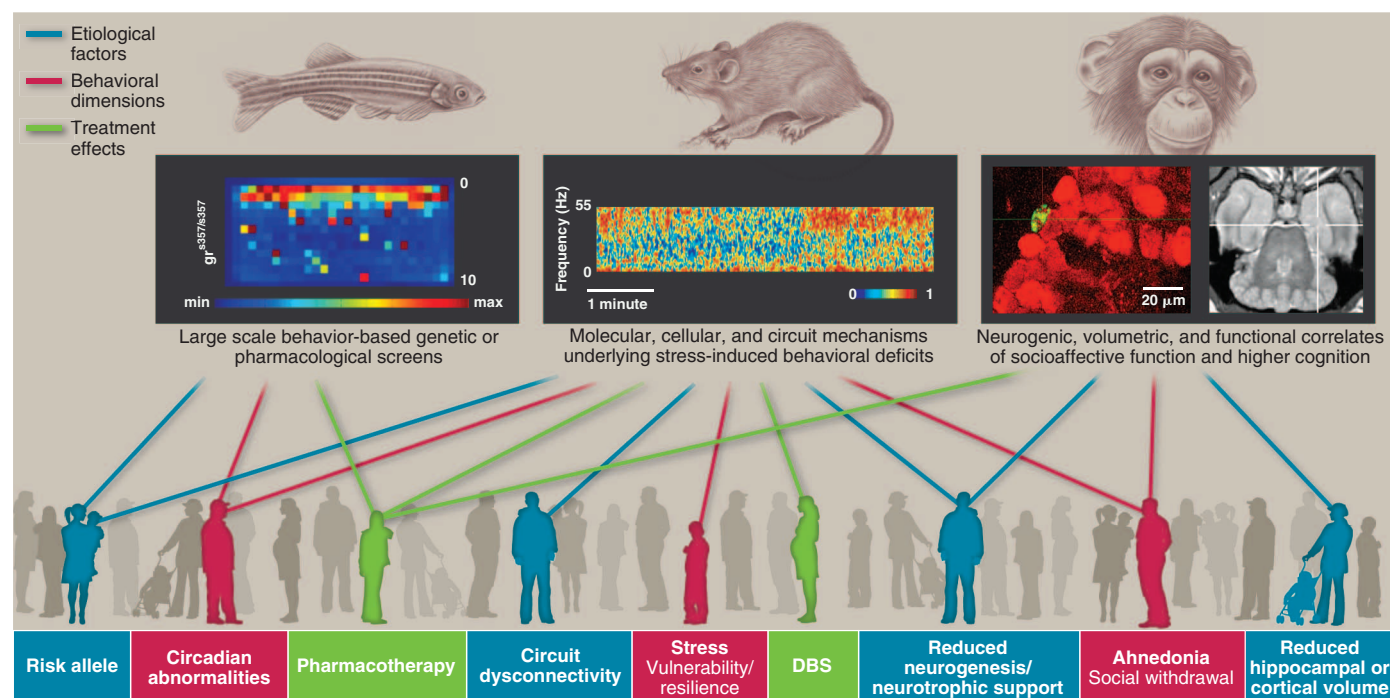


Fig. 1. Preclinical paradigms used in the study of MDD-related neurobehavioral traits in nonhuman species. No etiologically valid depression-like syndrome that reproduces the complexity and heterogeneity of the human syndrome has been established in nonhuman animals. Current paradigms capitalize primarily on exposure to acute or chronic stressors as a depressogenic trigger and examine species-specific behavioral repertoires in an attempt to capture distinct behavioral or psychological dimensions of the illness. Examples of animals studied include the zebrafish, various laboratory rodents (including rats and mice), and nonhuman primates. Models are used to inform the etiology of MDD by replicating environmental risk factors, circuit abnormalities, and genetic mutations affecting candidate biological pathways. Various models also replicate responses to pharmacotherapies or somatic therapeutic interventions, such as electroconvulsive therapy and DBS. The zebrafish is a model organism commonly used to dissect behaviorally relevant neuronal networks. It is amenable to large-scale phenotypic screens, generally based on automatic movement tracking (40, 41). This approach is illustrated here with a heat map depicting movement during a social interaction task in an adult zebrafish homozygous for the mutation gr^{357} , which disrupts tran-

scriptional regulation by glucocorticoid receptor and leads to abnormal socioaffective behaviors sensitive to antidepressants (42). A range of behavioral tasks is available in rodents to examine mechanisms underlying stress-induced motivational, affective, cognitive, and social deficits associated with MDD (5). Spectral plots derived from multicircuit field recordings depict changes in the oscillatory synchrony of the basal amygdala and nucleus accumbens during depression-related behaviors in the mouse (11). Nonhuman primates have provided the most immediate evidence for the occurrence of “pathological” deviations of socioaffective behaviors resembling depression in animals (23, 24) and are also providing key insights about higher cognitive dysfunctions associated with depression (35). A form of depression-like behavior, observed in both free-ranging and captive nonhuman primates, is a behavioral posture strikingly reminiscent of human prostrated sadness and social detachment, first described in macaques deprived of secure maternal attachment during infancy (60). A small number of studies have used primates to examine factors associated with pathophysiology [such as hippocampal volume (61) and treatment response such as regulation of hippocampal neurogenesis (25)] in the context of social stress.

notion of cognitive-affective bias, which derives from the observation that depressed patients over-emphasize negatively valenced information and have difficulty redirecting their attention, thoughts, or memory away from negative material (34). A recent study relying on a novel cost-benefit decision task in macaques recently identified a subregion of the anterior cingulate cortex that encodes negative motivational value and whose microstimulation shifts animal choices toward pessimistic predictions of outcomes (35). The fact that this subregion is contiguous and interconnected with *cg25* and is similarly regulated by various classes of antidepressants reinforces its possible role in MDD. As the use of analogous (albeit more rudimentary) behavioral tasks is beginning to be reported in rodents, it will be important to determine if the ventromedial prefrontal cortex in these species plays a similar role in determining “pessimistic” decision biases (36, 37).

Discovering new behavioral end points. As our understanding of circuit modules underlying MDD symptoms and therapeutic responses increases, and circuit-perturbation approaches (based on optogenetics (10), pharmacogenetics (38) and other methods) continue to mature and disseminate to reach a wider range of species including primates, we may be able to start forward-engineering “tunable symptoms” in animals that can support antidepressant therapeutic screens. In combination with “phenomics” tools—such as those allowing circadian monitoring of complex home-cage behaviors in groups of rodents (39) or high-throughput behavioral-based screening in zebrafish (40, 41)—these approaches may offer “unbiased” means to discover novel depression-related behavioral end points and to establish a cross-species taxonomy based on the genetic conservation of underlying circuits rather than on simple behavioral resemblance.

Toward Translational Models of Genetic Susceptibility and Cellular Dysfunction in Depression

In the search for etiologically valid animal models of MDD, much hope has been placed in recent years in our ability to harness the power of genomics to resolve the genetic architecture of MDD heritability (estimated to 40%) and to identify causal mutations that can be reproduced in animals to emulate (in interaction with stress inducers discussed above) various aspect of behavioral and neural MDD phenotypes.

The majority of etiologically valid mutant models have been so far developed in the context the “common variant” hypothesis, whereby the genetic risk for MDD is hypothesized to reflect the additive or multiplicative influence of several alleles with small effect. Several depression-like phenotypes have been reported in animals carrying mutations replicating naturally occurring, nonsynonymous single-nucleotide polymorphisms that alter the function of mainstream MDD candi-

date genes, such as *BDNF* (42), *TPH2* (43), or *5-HTT* (44). Other mouse lines have targeted potential risk genes that emerged from unbiased genome-wide association studies (GWAS), such as the presynaptic protein *Piccolo* (45).

Humanized animals that faithfully reproduce variants naturally occurring in patients have allowed the development of pioneering translational studies comparing human and animals along in analogous behavioral tasks [for review, see (46)]. Because they allow examination of the effect of human alleles in the context of a homogenous genetic background, in animals raised under controlled environments, humanized mutants such as the *BDNF* Val66Met knock-in mice represent a powerful approach to identify functional effects that may be difficult to replicate or even detect in clinical setting. Studies with these mice have provided convincing evidence that individual variations in affective traits that predispose to depression (such as threat perception or fear extinction) are influenced by variations in the trafficking and release of *BDNF* resulting from the substitution in a single codon (47). However, the epidemiological evidence that this variant significantly increases the MDD’s risk remains contentious for reasons that, as discussed above, may reflect the heterogeneous stratification produced by MDD diagnosis as much as the complexity of depression genetics.

As genotyping methods progress and the sizes of the cohorts included in GWAS for MDD continue to grow and facilitate the replication of small effect sizes, more stringently validated MDD risk genes [such as the recently identified *SLC6A15* gene that encodes a neuron-specific neutral amino acid transporter (48)] may become more frequent, ultimately opening the way to experimental studies examining how the penetrance of depression-related phenotypes is moderated through epistatic interactions in synergy with potent environmental constraints applied at specific developmental periods. Although tackling this question experimentally will require surpassing a number of technical barriers (such as the ability to simultaneously engineer a multiplicity of targeted point mutations in the same animal), we can look ahead optimistically given the rapid pace at which emergent genome editing methods (such as TALENs or zinc finger nucleases) are evolving. Because these approaches not only hold the promise of facilitating the generation of mutant animals but also widen the range of model species in which these mutations can be studied, they may be announcing the end of the upsurge of the mouse as a model species for preclinical MDD studies.

The lack of a highly penetrant mutation associated with MDD is often presented as one of the primary causes for the lack of valid MDD animal models. This may change given the growing evidence of a higher burden of copy number variants in depressed patients (49) (i.e., rare per-mutations in large portions of gene or chromo-

somes) and the early characterization of one such rare mutation with strong penetrance [a duplication recently reported in the *SLIT3* gene that encodes a protein involved in axon guidance (50)].

Another important line of data in this context involves the well-replicated, albeit not systematic, depression-related phenotypic features (i.e., in anhedonia and “despair” tests) reported in the several different lines of mutant mice now available for the *DISC1* gene (51). Translocation of *DISC1* in the Scottish kindred leads to a variegated phenotype (34% of carriers are diagnosed with MDD versus 24% with schizophrenia or bipolar disorder) and the link between *DISC1* and MDD has been reinforced by several recent lines of evidence for an association of *DISC1* with symptom severity and age of onset in MDD (52). The question of how various or even identical mutations of *DISC1* could increase risk to develop MDD versus other psychiatric disorders converges with the current debate that surrounds the evolution of psychiatric nosology. As we look ahead, the increasing availability of induced pluripotent stem cells derived from patients carrying *DISC1* mutations (53) may help tease apart relevant cellular mechanisms and facilitate reverse translation. Emergence of other *ex vivo* systems—such as olfactory neuroepithelial cell lines that can be biopsied both in patients and animals for longitudinal studies of various aspects of neural function and signaling—also offer unique translational opportunities to dissect the respective impact of genetics, epigenetics, and environmental factors on intracellular signaling mechanisms relevant to stress vulnerability and risk for MDD (54).

In part thanks to the development of tissue-bank initiatives, postmortem transcriptomics and proteomics have helped link regional evidence of neuropathology in MDD with abnormalities in a number of candidate signaling pathways that are now extensively studied in animals. These results continue to involve neurotrophic signaling cascades (55) and neurogenic processes, two inter-related mechanisms mediating the therapeutic activity of monoaminergic antidepressants. Regulation of monoaminergic and amino acid neurotransmission, neurohormonal signaling, and inflammatory cascades continue to be other active areas of investigation for MDD cellular pathogenesis (56). Progress of conditional and inter-sectional genetic strategies and virally mediated manipulations have allowed dissection of these pathways in animals with greater degrees of precision, both in term of cell type and temporal specificity. Advances in these strategies have helped pinpoint critical periods for the developmental programming of emotional functions relevant to MDD (57) and have allowed identification specific cell-types by which important modulators of stress vulnerability such as *CRHR1* (12), *P11* (58), and *HDAC6* (30) exert their effects on behavior.

Despite these important new leads, there is unfortunately still no consensus about a bona fide genetic mouse model of depression, with a well-replicated, multifaceted phenotype and strong pharmacological validity, at par, for instance, with those used for the study neurodegenerative disorders. This could reflect the unique complexities of MDD etiology and phenomenology, but may also derive, to a certain extent, from our lack of concerted efforts to homogenize behavioral paradigms and methodologies across laboratories. This is a likely cause for the frequent lack of replication of Gene $\times$ Environment effects and an issue that will become increasingly important as studies of epigenetic mechanisms in models of depression become more common (59). Another critical lack of consensus is related to the time course of antidepressant responses in animal models: Although a delayed response requiring weeks of treatment might simulate the clinical effects of current antidepressants, this question needs reconsideration given the emergence of the potential fast and long-lasting antidepressant effects of ketamine and derivatives (14). There is a need to determine how the doses of drugs administered to animals relate to their clinical effects for future discoveries, because brain or blood levels of drugs necessary for the engagement of target effects are rarely reported in animal studies. Although a large number of patients remain resistant to current treatments, there is little consensus on how to develop animal models for these subpopulations.

Conclusion

Neuroscience may not hold all the keys to a public health issue as complex as depression. However, the tremendous advances made over the past few decades continue to hold the promise that a better understanding may ultimately ease suffering and erase stigma. Animal models are pivotal in this effort to translate basic progress into better care. The brief overview proposed here suggests that, although it seems unlikely that any one model will ever recapitulate this heterogeneous illness in its entirety, many current paradigms are yielding key neurobiological insights relevant to behavioral dimensions and affective constructs in humans. A challenge remains to understand how these dimensions integrate in the context of pathology. A second challenge will be to effectively align variables measured in animals with those assessed in genetic studies or during the various phases of development of novel antidepressants. Translational medicine is a two-way bridge. Preclinical research needs to inform clinical trials and diagnosis, but the reverse is also true. Without a consensus about what depression is and how to more reliably and specifically measure it, rapid progress seems unlikely. Thus, it is critical to keep in mind the conceptual, methodological, and organizational factors that cause the field of depression therapeutics to remain at least one step behind in the pursuit of valid animal models.

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PERSPECTIVE

The Science of Resilience: Implications for the Prevention and Treatment of Depression

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Human responses to stress and trauma vary widely. Some people develop trauma-related psychological disorders, such as posttraumatic stress disorder (PTSD) and depression; others develop mild to moderate psychological symptoms that resolve rapidly; still others report no new psychological symptoms in response to traumatic stress. Individual variability in how animals and humans respond to stress and trauma depends on numerous genetic, developmental, cognitive, psychological, and neurobiological risk and protective factors.

Resilience to stress is a complex multidimensional construct. Although there is no one universally accepted definition of resilience, it is generally understood as the ability to bounce back from hardship and trauma. The American Psychological Association defines

resilience as “the process of adapting well in the face of adversity, trauma, tragedy, threats or even significant sources of threat” (1).

Genetic factors play an important role in an individual’s response to stress and trauma (2). Twin studies have estimated an overall heritability

of posttraumatic stress disorder (PTSD) ranging from 32 to 38%, and DNA studies have found that regulation of the stress response is affected by genetically mediated differences in reactivity of the sympathetic nervous system (SNS) (e.g., polymorphism of the alpha-2C adrenergic receptor gene), the hypothalamic-pituitary-adrenal axis (HPA axis) [e.g., functional variants of brain mineralocorticoid, glucocorticoid and corticotropin-releasing hormone (CRH) receptor genes], neuropeptide Y (NPY), and the serotonin system, among others.

The best-studied gene-environment interaction involves a naturally occurring variation in the promoter of the human serotonin transporter gene (*5-HTTLPR*) (3, 4). The short allele of *5-HTTLPR* and a single base substitution in the long form of *5-HTTLPR* are associated with decreased serotonin transporter availability and a resulting lower reuptake of serotonin from synaptic clefts. It appears that these lower-expressing alleles may be specifically associated with an increased risk of depression following exposure to childhood maltreatment (5). It has been proposed that the mechanism underlying the gene-environment interaction between the serotonin transporter gene and stress may involve alteration in the critical amygdala-ventromedial prefrontal cortex (PFC) and dorsal raphe circuitry that is similar to that observed in depressed patients (6).

Developmental risk and protective factors have an enormous impact on brain development and on shaping neural circuits that regulate future responses to stress and adversity. Repeated episodes of uncontrollable or overwhelming stress during infancy and childhood, such as child abuse, can lead to “learned helplessness” and can cause exaggerated emotional, behavioral, SNS, and HPA-axis responsiveness to future stressors, even into adulthood (2, 7, 8).

On the other hand, mild-to-moderate stressors that are controlled and mastered can have a “steeling” or stress-inoculating effect, where the child develops an adaptive stress response and becomes more resilient than normal to the negative effects of future stressors. In animal studies, early experiences with successful behavioral control over stressful events induce neuroplasticity in the PFC, which appears to protect the animal from some of the negative effects of future uncontrollable stress. An enriched environment and consistency of supportive maternal care provide an atmosphere that fosters exposure to novelty

and the mastering of challenges. Negative and positive neurobiological and behavioral consequences of parental care can even be transmitted across generations, possibly through epigenetic mechanisms (9).

In addition to genetic and developmental factors, numerous neurobiological factors and systems mediate and/or moderate resilience to stress, including an HPA axis that is well modulated by dehydroepiandrosterone, NPY, and other regulators of CRH activity; a SNS that responds effectively to stress and provocation, but that returns to baseline rapidly secondary to regulation by NPY and galanin; a mesocorticolimbic dopaminergic-mediated reward system that is durable and that maintains positive emotions and/or optimism in the face of acute and chronic stress; functional hippocampi that adequately inhibit the HPA-axis response to stress and that have the ability to differentiate dangerous versus safe environments; robust PFC executive functioning and capacity to inhibit and regulate limbic, emotional, and behavioral reactivity to stress; and well-modulated amygdala activity that does not over- or underreact to external or internal stimuli (2).

Psychosocial factors that have been associated with resilience include positive emotion and optimism, loving caretakers and sturdy role models, a history of mastering challenges, cognitive flexibility including the ability to cognitively reframe adversity in a more positive light, the ability to regulate emotions, high coping self-efficacy, strong social support, disciplined focus on skill development, altruism, commitment to a valued cause or purpose, capacity to extract meaning from adverse situations, support from religion and spirituality, attention to health and good cardiovascular fitness, and the capacity to rapidly recover from stress (10).

Utilization of these resilience-promoting factors can be beneficial throughout the life span. Most research has shown that older adults tend to be more stress-resilient than younger adults. Potential contributing factors include prior experience with trauma and stress inoculation, more mature and effective coping styles, greater acceptance of and tolerance for negative affect, and better regulation of emotions. In older adults, resilience has been associated with social connectedness, curiosity, spiritual grounding, and wisdom (11).

Resilience-Informed Strategies and Interventions for Prevention and Treatment of Depression

How can what we currently know about resilience be applied to the prevention and treatment of depression? There are several areas that have been studied.

Genetics and environment. Research in genetics and epigenetics suggests that putative vulnerability genes or “risk alleles” operate in a

dynamic interplay with the environment and that resilience may be promoted, in some cases, by changing the biological and/or psychosocial environment (12). For example, in a study of maltreated children, positive social support appeared to protect against depression, even in children having the short allele of the serotonin transporter gene (13).

Child rearing. To protect against learned helplessness and depression, as well as to promote resilience, it is critical to provide children with a supportive and loving environment that fosters healthy attachment, protects them from repeated experiences of uncontrollable stress, and provides them with ample opportunities to master life challenges. Such mastery can contribute to stress inoculation with reduced overall reactivity to future stressors and enhanced mastery of future challenges. Classes in effective parenting might help to provide a resilience-promoting child-rearing environment and to reduce transgenerational transmission of stress vulnerability.

Social support. Low levels of social support have been associated with depression, PTSD, and medical morbidity, whereas high levels of social support have been positively associated with active problem-focused coping, sense of control and predictability in life, self-esteem, motivation, optimism, enhanced immune function, dampened neuroendocrine and cardiovascular responses to stress, resilience, and lower levels of depression. Interventions that teach children and adults the skills needed to improve social competence and to construct and maintain supportive social networks are likely to enhance resilience and to decrease rates of stress-related depression. Social-emotional training programs for children, which focus on enhancing executive function and prosocial behavior, have shown promise in strengthening social skills, social networks, and academic performance (7).

Cognitive and/or psychological interventions. When individuals believe that the demands of a stressful situation exceed their personal capabilities and external resources, they tend to appraise the situation as a threat and as out of their control, which negatively affects their emotional and behavioral response and increases the likelihood of developing depression. On the other hand, if the individual believes that they have the skills, experience, and resources needed to successfully deal with an adverse situation, they are more likely to appraise the situation as a challenge.

A number of therapeutic approaches have been designed to modify appraisals of threat and adversity (14, 15). These include training in attention control, cognitive reappraisal, and enhancing self-efficacy. Interventions in attention control, such as cognitive control training and mindfulness training, teach individuals how to control where they direct their attention and have shown promise as treatments for depression. Learning

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to selectively attend to positive as well as relevant negative information, while filtering out irrelevant negative information (i.e., consistent with realistic optimism, a pattern that has been associated with resilience), would be particularly helpful for the pessimistic or depressed individual who tends to preferentially focus on, remember, ruminate about, and have difficulty disengaging from negative information. Deficient top-down cognitive control and/or PFC inhibition of subcortical brain regions—such as the amygdala, dorsal raphe nucleus, and habenula—might perpetuate depression through reduced capacity to regulate stress-related emotions and by contributing to negative biases (8, 16).

Cognitive reappraisal, or the ability to cognitively reframe adverse and negative events in a more positive light, is strongly associated with resilience and can moderate the relation between severity of life stress and depression (14). It may accomplish this, in part, by attenuating negative emotional and biological stress responses (7, 8, 16). Interventions that employ training in cognitive reappraisal and that have shown some promise in reducing depression include well-being therapy (17), hardiness training (18), and Viktor Frankl’s logotherapy. Searching for and extracting meaning, purpose, and strength from adversity is an important component of these therapies. Many studies have shown that having a highly valued and meaningful purpose or mission can enhance resilience to stress.

Training in cognitive reappraisal is also a central component of many cognitive-behavioral therapies, which are well-established and effective

treatments for depression and PTSD. These therapies typically teach individuals to observe their cognitive and behavioral reactions to stress, to challenge distorted negative appraisals of self and the situation, and to replace distortions with more realistic, accurate, and positive appraisals.

Coping self-efficacy refers to perceived capacity to successfully manage and recover from the demands of a stressful situation. High coping self-efficacy is highly predictive of resilience and adjustment after traumatic stressors such as military combat, motor vehicle accidents, death of a spouse, and natural disasters (10, 15). There are many ways to increase coping self-efficacy. One of the most important involves mastery experiences, where the individual learns the skills needed to successfully manage a stressor and then practices those skills, preferably with feedback, in increasingly challenging situations until he or she has mastered the challenge. Having confidence in one’s capacity to deal with stress may increase a sense of control, shift a perceived threat into a perceived challenge, foster active problem-oriented coping, increase motivation and perseverance, modify emotional and neurobiological responses to stressors, and buffer against stress-related psychological disorders such as depression. Training programs designed to enhance mastery and coping self-efficacy in stressful situations are typical of military, police, and firefighter training, as well as outdoor education programs, like Outward Bound.

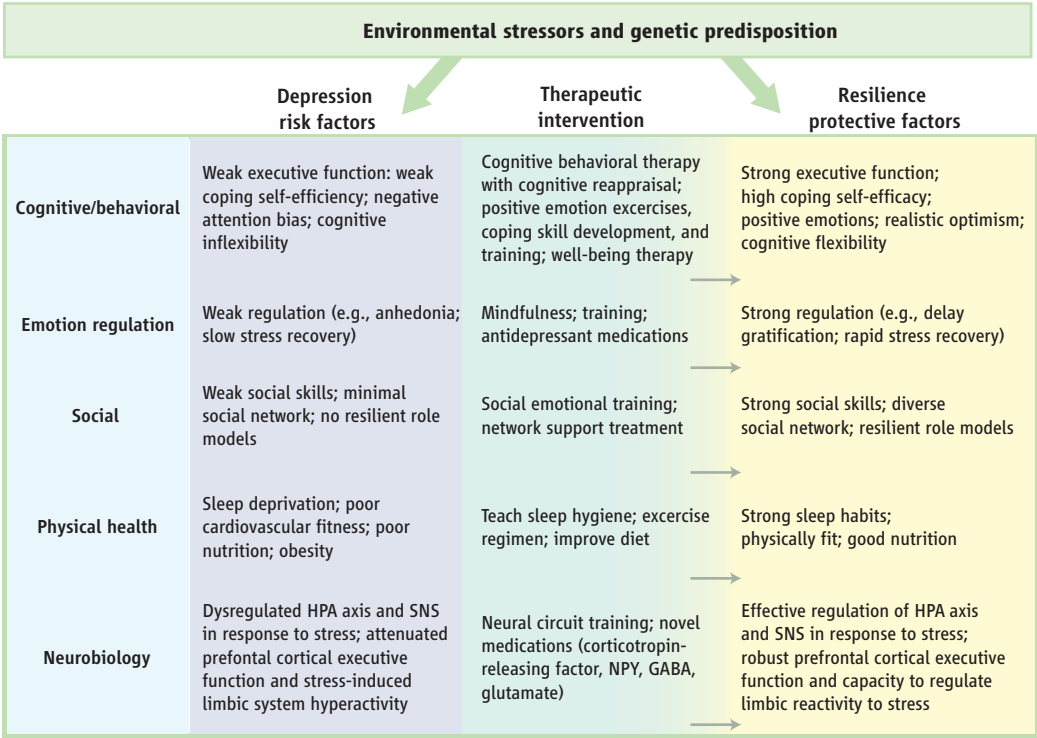
Neurobiological interventions. A better understanding of the neurobiology of resilience will hopefully lead to prevention and/or improved treatment

for stress-related disorders such as depression and PTSD (2). For example, enhancing NPY function, particularly for individuals who do not naturally release sufficient amounts, might boost physiological resilience by helping to maintain the SNS and HPA axis at an optimal level of activation—high enough to respond to danger but not so high as to stimulate excessive fear, anxiety, and depression. Similarly, developing therapeutic agents to contain stress-induced overdrive of CRH, which controls and integrates the body’s response to stress, would likely reduce rates of trauma-related psychopathology.

Other mediators of stress resilience that could serve as therapeutic targets for reducing the likelihood of developing stress-related depression include the serotonin, dopamine, noradrenergic, γ -aminobutyric acid, and glutamate systems. For example, antidepressants protect against stress-induced learned helplessness in animals and stimulate the regrowth of hippocampal neurons that have been damaged by stress, and α -adrenergic agents, like propranolol, may have a role in preventing overconsolidation of traumatic memories. It may also be possible to develop pharmacological and/or psychotherapeutic interventions to help regulate neural pathways believed to be critical to resilience, including pathways involved in emotion regulation, attention, positive versus negative outlook, reward and motivation, sensitivity to context, response to fear, learning and memory, adaptive social behaviors, and speed of recovery from stress.

Real-time functional magnetic resonance imaging neurofeedback, mental training exercises, mindfulness meditation, and cognitive reappraisal training are exciting new directions of research that target top-down regulation of specific neural circuits. For example, mindfulness meditation and cognitive reappraisal are believed to exert their adaptive effects on emotion regulation by enhancing PFC regulation of limbic and brainstem systems. It is possible that therapies designed to stimulate and strengthen PFC regulation of emotion will boost confidence in one’s ability to gain control of stressful situations (7, 8). To increase resilience and decrease rates of stress-related depression, it will also be important to develop and test interventions that target bottom-up regulation of emotion (8).

Improving physical health. Quality of diet, amount of exercise, capacity to relax, and quantity and quality of sleep are important in determining how the body and brain respond to stress (19). For example, aerobic exercise has been associated with resilience largely through its



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effect on reducing anxiety and depression and improving cognition and brain function. Regular aerobic exercise is believed to induce the expression of genes associated with neuroplasticity and neurogenesis, as well as to regulate the HPA-axis response to stress. In some studies, exercise was as effective as antidepressants in treating mild-to-moderate depression and may possibly protect against future episodes of depression (10, 19)

More comprehensive resilience training programs. Although far more research is needed to determine their efficacy, examples of resilience training programs that employ several of the above cognitive behavioral and emotion regulation strategies include hardiness training (18), The Penn Resiliency Program (20), and the military's Battlemind or Comprehensive Soldier Fitness program (21).

Implications. What we currently know about resilience has implications for the prevention and treatment of depression. Risk and protective factors generally have additive and interactive effects so that having multiple genetic, developmental, neurobiological, and/or psychosocial risk factors will increase allostatic load or stress vulnerability, whereas having and enhancing multiple protective factors will increase the likelihood of stress resilience (19). Because neuroplasticity is exhibited throughout the life span, many of the stress-protective factors described

in this Perspective can be enhanced through practice and training, which make it possible to improve adaptation to stress, increase speed of recovery from stress, and decrease the chances of developing stress-related depression throughout the life span. However, interventions that are initiated early in development are likely to have the greatest impact on future stress resilience, as there appear to be time-limited windows of enhanced neuroplasticity. Nevertheless, recent research suggests that it may be possible to open or reopen windows of neuroplasticity, perhaps with drugs, which might then enhance the efficacy of behavioral interventions (19). The study of resilience and its neurobiological underpinnings is a relatively young area of scientific investigation. Similarly, many approaches to enhancing resilience are still in experimental stages. Advances in our understanding of resilience and of its association with depression will come from continued multidisciplinary research on complex interactions between genetic, developmental, neurobiological, and psychosocial risk and protective factors. It is anticipated that this knowledge can then be used to inform the development of evidence-based interventions to mitigate risk for depression and to enhance resilience to stress.

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The Shortest-Known-Period Star Orbiting Our Galaxy's Supermassive Black Hole

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Stars with short orbital periods at the center of our Galaxy offer a powerful probe of a supermassive black hole. Over the past 17 years, the W. M. Keck Observatory has been used to image the galactic center at the highest angular resolution possible today. By adding to this data set and advancing methodologies, we have detected S0-102, a star orbiting our Galaxy's supermassive black hole with a period of just 11.5 years. S0-102 doubles the number of known stars with full phase coverage and periods of less than 20 years. It thereby provides the opportunity, with future measurements, to resolve degeneracies in the parameters describing the central gravitational potential and to test Einstein's theory of general relativity in an unexplored regime.

The recent advent of high-resolution imaging capabilities on large ground-based telescopes has afforded us a view of the center of our Galaxy that is substantially altering our understanding of this region and by analogy the centers of other galaxies. The earliest high-resolution images in the near-infrared (NIR) revealed the presence of a black hole [Sagittarius A* (Sgr A*)] with a mass 4×10^6 times that of the Sun by detecting stars at the very heart of the Galaxy, the so-called S-stars, with velocities of up to $\sim 10,000$ km/s (1, 2). The detection of accelerations in the motion of these stars (3, 4) further strengthened the case for a nonstellar dark mass at the center of the Galaxy. After it passed periastron (the closest point to the black hole in the orbit of a star), a Keplerian orbit could be determined for the star S0-2 (with an orbital period of 16 years), proving the black hole hypothesis beyond reasonable doubt (5, 6). This star can now be traced in its motion around the black hole with such precision and complete phase coverage that its three-dimensional (3D) orbit gives the most accurate description to date of the central mass and the distance from our solar system to the center of the Galaxy (7–9). No other star has so far been reported with more than $\sim 40\%$ of its orbit covered by data.

Here we report the detection of another star, S0-102, in orbit around the supermassive black hole at the center of our Galaxy (10). It has an orbital period of 11.5 years, which makes it the shortest-period star known yet.

The data sets that we used for this study come from high-resolution imaging observations of the galactic center that were obtained at the W. M. Keck Observatory between 1995 and 2012 with speckle imaging and adaptive optics (AO). Speckle imaging with the NIR imaging camera NIRC (11, 12) on Keck I provided the earliest high-angular-resolution imaging data sets (1995 to 2005), which are reported with their observational setup in several earlier papers (2, 3, 7, 13, 14). In summary, several thousand data frames capture images of the galactic center on time scales of ~ 0.1 s, which is short enough compared to the coherence time of atmospheric turbulence to freeze the distorting effects of Earth's atmosphere and preserve the source's information at all spatial frequencies transmitted by the telescope. Table S1 summarizes key observational information for the 26 epochs of speckle imaging data that are available.

AO observations of the galactic center were carried out between 2004 and 2012, with substantially more data per epoch being obtained in 2006 and onward. Previous publications contain the observational details for the first nine epochs of AO observations (7, 14–16). We collected 12 further epochs of AO observations between May 2008 and May 2012, using an observational setup identical to those for all AO observations obtained in 2006 and onward. For all but two epochs, the high-order AO compensation was based on observations of a bright [R band ~ 10 magnitudes (mag)], on-axis, laser guide star, generated with the Keck II sodium laser; and the low-order (tip-tilt) AO corrections were deter-

mined from measurements of the natural guide star USNO 0600-28577051 ($R = 13.7$ mag and $\Delta r_{\text{Sgr A}^*} = 19''$) ($\Delta r_{\text{Sgr A}^*}$, distance to Sgr A*). Table S2 provides the details of the 20 AO epochs that are used here.

The data analysis for this study falls into several distinct stages (17). First, for each epoch of observations, we constructed an average image of all the high-quality data collected as well as three subset images, each composed of one-third of the data (see Fig. 1 for a zoom into the region of an AO image where S0-102, S0-2, and the electromagnetic manifestation of the black hole, Sgr A*, reside). Second, both previously known and newly detected stars were identified in the images, along with their photometric and astrometric characteristics, with the point spread function (PSF) fitting program StarFinder (18). Third, the positions of the stars in each epoch were transformed into a common coordinate system. Fourth, orbital model fits to the positions of stars with significant orbital phase coverage provided estimates of each star's orbital parameters.

The gravitational potential in this region of the Galaxy is dominated by the black hole, and to a good approximation the stars follow Keplerian orbits. Although deviations from a pure Keplerian orbit will probably be detected in the future, this cannot be expected with the current data set, and we therefore assumed a point-mass potential and Newtonian gravity. Within this framework, we

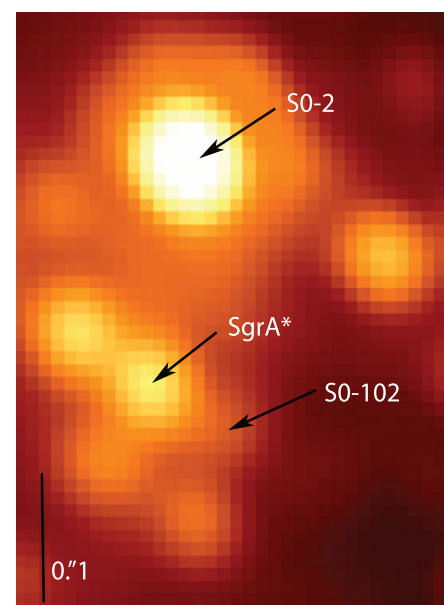


Fig. 1. A Keck/NIRC2 AO image from May 2010 showing the short-period star S0-102, which is, besides S0-2, the only star with full orbital phase coverage, and the electromagnetic counterpart of the black hole, Sgr A*. The image was taken at a wavelength of $2.12 \mu\text{m}$ and shows the challenge of detecting S0-102, which is 16 times fainter than S0-2 and lies in this crowded region.

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used a χ^2 -minimization routine to find the best-fit orbital parameters (7). The six orbital elements describing the star's trajectory are the period, eccentricity, time of closest approach (periapse passage), inclination, angle to periapse, and angle of the ascending node. The seven parameters describing the gravitational potential are mass, 3D position (distance from Earth and focal point of the orbital ellipse) and 3D velocity of the point mass.

We have covered a complete orbit of S0-102 with astrometric observations. Therefore, the data contain enough information that they can in principle be used to fit for both the star's orbital elements and also for the parameters of the potential (19). However, this requires the same low level of systematic effects in the data as for S0-2, which dominates our current knowledge about the potential and is a factor of 16 brighter than S0-102. Considering that fainter sources are much more prone to source confusion

than brighter ones, it is unlikely that S0-102 has the same low level of systematic errors (7, 8).

A way of minimizing the effects of possible confusion is to not fit for the potential, but rather fix it to the values that have been derived from the orbit of S0-2 (table S4). This left only S0-102's orbital elements free, which were then found by fitting the data (Fig. 2). Overall, the data are well fit by this model, which has a reduced χ^2 of 2.0. The most discrepant points are the two epochs in 2009, both of which lie 2.3σ away from the best fit. This is the region around closest approach to the black hole, where Sgr A* and S0-104 (20), which are blended in this epoch, are very close [~ 55 milli-arc sec (mas)] to S0-102. This constellation most likely leads to an astrometric bias, which supports our conservative approach of fixing the parameters of the potential.

We determined uncertainties on S0-102's orbital parameters via Monte Carlo simulations as-

Fig. 2. The orbits of S0-2 (black) and S0-102 (red). RA, right ascension; DEC, declination. The data points and the best fits are shown. Both stars orbit clockwise. The dashed lines represent the parts of the orbits that have been observed with Speckle data; the solid lines indicate AO observations. The data points for S0-2 range from the year 1995 to 2012, and S0-102's detections range from 2000 to 2012. The connecting lines to the best fit visualize the residuals. Although the best-fit orbits are not closing, the statistically allowed sets of orbital trajectories are consistent with a closed orbit. S0-102 has an orbital period of 11.5 years, which is 30% shorter than that of S0-2, the shortest-period star previously known.

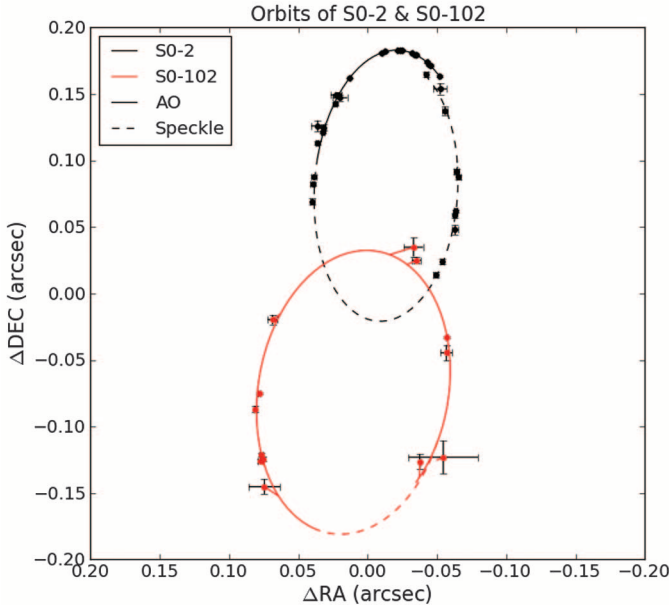


Table 1. Orbital elements for S0-102. The best fit has a χ^2 of 39.96 with 20 degrees of freedom.

Parameter	Value
<i>S0-102's orbital parameters</i>	
Period	11.5 ± 0.3 years
Time of closest approach (calendar year)	2009.5 ± 0.3
Eccentricity	0.68 ± 0.02
Inclination*†	$151^\circ \pm 3^\circ$
Angle to periapse	$185^\circ \pm 9^\circ$
Position angle of the ascending node†	$175^\circ \pm 5^\circ$
<i>Parameters of the gravitational potential‡</i>	
Mass	$4.1 \pm 0.4 \times 10^6 M_{\text{Sun}}$
Distance	7.7 ± 0.4 kpc

*90° is edge-on, and 0° is face-on. †The allowed ranges for inclination and angle of the ascending node are (0°, 180°). ‡The parameters that describe the gravitational potential have been taken from S0-2's orbit. We list mass and distance here; see table S4 for the 2D position and 3D velocity of the central mass.

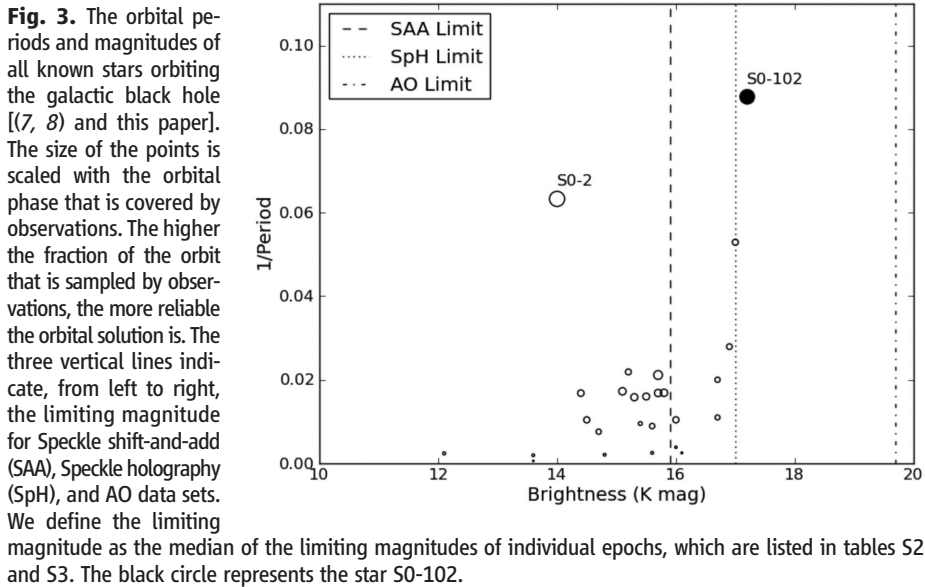
suming Gaussian error statistics. Using the best fit and the error bars on the measured data points, we generated 10^5 artificial data sets and fitted an orbit to each realization. The probability distribution functions for the orbital elements are the distribution of best-fit values from the Monte Carlo simulations (Table 1). This approach assumes that the positional errors are statistical in nature and does not account for possible systematic contributions such as unrecognized source confusion.

Our high-angular-resolution imaging campaign of the galactic center started in 1995, constituting a time baseline of 17 years, 7 of which were carried out with deep AO observations. Although this is long enough to detect accelerations (i.e., curvature) of several stars within the central arc second, it allows a reliable orbit determination only for the shortest-period stars. We consider an orbit reliable if it is likely that an increase in data and a decrease in noise do not lead to major changes in the orbital elements. As a rule of thumb, 50% of the orbit needs to be covered by observations in order to determine the orbit reliably (21). Our data set traces S0-102 through a complete orbit, making it the only star other than S0-2 for which such a fraction of the orbit has been sampled. The key property that enabled this orbital coverage is S0-102's period.

With an orbital period of 11.5 years, S0-102 has the shortest period among all known stars orbiting the black hole in our Galaxy's center (Fig. 3). It is 5 years ($\sim 30\%$) shorter than S0-2's orbital period, which was the previously known shortest-period star. The period (or, alternatively, the semi-major axis, which is related to the period via Kepler's law) is the most important orbital parameter, because it not only makes it possible to sample a substantial fraction of the orbit by observations, but also simplifies the detection of relativistic effects, most of which are cumulative and build up with increasing phase coverage until they breach the detection threshold.

A test of Einstein's theory of general relativity is the next goal in galactic center research, now that the existence of a black hole is well established. This theory has so far passed all tests on solar system scales with flying colors. However, the gravitational potential ϵ of the Sun is very weak, with typical experiments probing regimes of up to $\epsilon \sim GM/(Rc^2) \sim 10^{-6}$. Here, G is the gravitational constant, M the mass scale, R the distance scale, and c the speed of light (22). The gravitational fields that have been probed in tests using double neutron stars such as the Hulse-Taylor binary pulsar are of the same magnitude, because the masses and separation of the neutron stars are comparable to the mass and radius of the Sun (23). In the galactic center, stars such as S0-102 and S0-2 probe gravity regimes that are two orders of magnitude stronger, $\epsilon \sim 10^{-4}$.

The effects of curved spacetime manifest themselves in two ways: The orbit of a star deviates from its Keplerian approximation, and



the path and wavelength of the light emitted by it get changed. It has been shown that for S0-2, the relativistic redshift should become measurable at S0-2's next periapse passage in 2018 (24, 25). Although this is a measurement of S0-2's redshift, S0-102 will render this measurement more precise. The reason for this is that, with AO observations of S0-102's apoapse passage in the next years, there will be enough information in the data set to lower the level of systematic effects to an insignificant level, and thereby allow S0-102 to be used to constrain the central gravitational potential. This additional information will then translate into a more precise determination of S0-2's orbital elements and therefore redshift measurement.

The relativistic redshift will also become observable in S0-102 itself. For S0-102, the difference between the purely Newtonian and fully relativistic Doppler shifts amounts to ~ 90 km/s. This is within the capabilities of upgraded or next-generation NIR spectrographs for sources of this magnitude. Because the Keplerian orbital parameters must be known precisely to infer the relativistic contribution to the redshift, only stars with well-sampled astrometric orbits can be used to detect deviations from a Newtonian orbit. Additionally, the relativistic redshift is strongest at periapse passage, and S0-2 and S0-102 are the only known stars that will pass periapse within the next 10 years and will have an orbital phase coverage greater than 50% then. Because S0-102 will pass closest approach 3 years after S0-2, an observation of its relativistic redshift will provide a crucial check on systematic effects in S0-2's result and test the independence of the gravitational redshift from the interior structure of the star, which is a key assumption of general relativity.

Other than the gravitational redshift, the leading-order general relativistic effect is the precession

of the periapse within the orbital plane (26–31). This is a cumulative effect that adds up from orbit to orbit and is therefore much more likely to be detected in stars that complete several orbits during the time scale of an observational campaign. Angelil *et al.* (30) show that the effect of periapse precession scales as period^{-1} , quantifying the significance of a short period for the test of general relativity in an unprecedented regime.

The periapse precession, which leads to rosetta-shaped orbits, consists of two components: a general relativistic part that leads to a prograde precession, and a Newtonian part that leads to a retrograde precession. The Newtonian deviation of a Keplerian orbit is caused by the inevitable presence of extended dark mass around the black hole, which means that the gravitational potential is not pointlike (27, 31, 32). This extended mass distribution is thought to mainly consist of stellar remnants—stellar-mass black holes and neutron stars—supplied by the dense nuclear star cluster (33, 34). In order to measure the general relativistic part only, the detection of the periapse shift in more than one star is required to break the degeneracy. This makes a star such as S0-102 a necessary ingredient for a measurement of the warping of spacetime around a supermassive black hole.

Given the astrometric precision with which a star can be located on the detector (~ 0.1 mas and ~ 1 mas for S0-2 and S0-102, respectively) and with which a stable absolute reference frame can be constructed (currently ~ 0.1 mas/year), the precession of both S0-2's and S0-102's apoapse, which amounts to ~ 1 mas and ~ 0.3 mas, respectively, is probably only detectable with the next generation of telescopes such as the Thirty Meter Telescope. In this upcoming era, S0-102 will play as dominating a role as S0-2 plays in today's Keck era.

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that the summit of Mauna Kea has always had within the indigenous Hawaiian community. We are most fortunate to have the opportunity to conduct observations from this mountain. The data described in the paper are presented in the supplementary materials.

Supplementary Materials

www.sciencemag.org/cgi/content/full/338/6103/84/DC1
Materials and Methods
Supplementary Text
Figs. S1 and S2

Tables S1 to S5
References (35–42)

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Imaging the Homogeneous Nucleation During the Melting of Superheated Colloidal Crystals

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The nucleation process is crucial to many phase transitions, but its kinetics are difficult to predict and measure. We superheated and melted the interior of thermal-sensitive colloidal crystals and investigated by means of video microscopy the homogeneous melting at single-particle resolution. The observed nucleation precursor was local particle-exchange loops surrounded by particles with large displacement amplitudes rather than any defects. The critical size, incubation time, and shape and size evolutions of the nucleus were measured. They deviate from the classical nucleation theory under strong superheating, mainly because of the coalescence of nuclei. The superheat limit agrees with the measured Born and Lindemann instabilities.

Crystal melting is of considerable fundamental and practical importance to science and technology. Yet, our understanding of it is still rather incomplete. Thermodynamics reveals to us the equilibrium phases, but the kinetics of phase transitions have proved difficult to predict (1–3). Crystals melt heterogeneously from surfaces or grain boundaries once they are heated to the melting point (3, 4). By suppressing surface melting (5–8), a single crystal can be superheated to temperatures above its melting point. This metastable state will eventually melt from the interior without any preferential sites. In such homogeneous melting, small liquid nuclei form spontaneously by thermal fluctuations. A spherical nucleus has the free energy (9)

$$\Delta G = 4\pi r^2 \gamma - \frac{4}{3} \pi r^3 n \Delta \mu + E_{\text{strain}} \quad (1)$$

where r is the radius, γ is the surface tension, n is the number density of the nucleus, $\Delta \mu$ (>0) is the chemical potential difference between the superheated crystal and liquid, $E_{\text{strain}} = \frac{4}{3} \pi r^3 n \Delta \epsilon$ is the misfit strain energy in the crystal caused by the volume change of the nucleus, and $\Delta \epsilon$ is the mean strain energy per particle from continuum elasticity and is not expected to depend on r . E_{strain} is zero when the parent phase is fluid but is finite when the parent phase is solid (10). To minimize ΔG , small liquid nuclei tend to recrystallize rather than grow unless their size exceeds a critical value $r^* = 2\gamma/[(\Delta \mu - \Delta \epsilon)n]$ corresponding to the barrier height of ΔG .

The small length and time scales of the nucleation process preclude observation at the single-particle level in molecular systems. In contrast, micrometer-sized colloidal particles can serve as good model systems for phase transition studies because their thermal motions can be directly visualized and measured with video microscopy (11, 12). Crystallization (13), sublimation (14), and heterogeneous melting in polycrystals (4, 15) have been studied in colloids.

We used thermal-sensitive *N*-isopropylacrylamide (NIPA) microgel colloidal spheres (4), whose effective diameter σ linearly changes from 0.76 μm at 26.4°C to 0.67 μm at 30.6°C (10). The effective diameter is defined so that the melting volume fraction $\phi_m = 54.5\%$, which is the same as that of the hard spheres. By this definition, the measured freezing point $\phi_f = 49\%$, which is close to that of the hard spheres ($\phi_f^{\text{HS}} = 49.4\%$) (16). At $49\% < \phi < 54.5\%$, crystal and liquid coexist. The volume fraction in colloids plays a similar role to the temperature in molecular systems (16). Particles were loaded into an 18 by 3 by 0.1 mm^3 glass channel and annealed to a single-face-centered cubic crystal or a polycrystal with only a few domains. The crystalline structure and the good refractive-index matching between particles and water enable us to see through all the 150 layers by means of bright-field microscopy (4). By changing the temperature, we can easily change the volume fraction to melt or recrystallize the sample repeatedly. However, because melting always starts heterogeneously from surfaces for a single crystal and from grain boundaries for a polycrystal (15), to create homogeneous melting the interior of a perfect crystalline domain was heated with a beam of light passing through an objective lens (Fig. 1A and movie S1) (10). The 4500- μm^2 heated area in the focal plane had a temperature $T_{\text{amb}} + \delta T$, where the ambient tem-

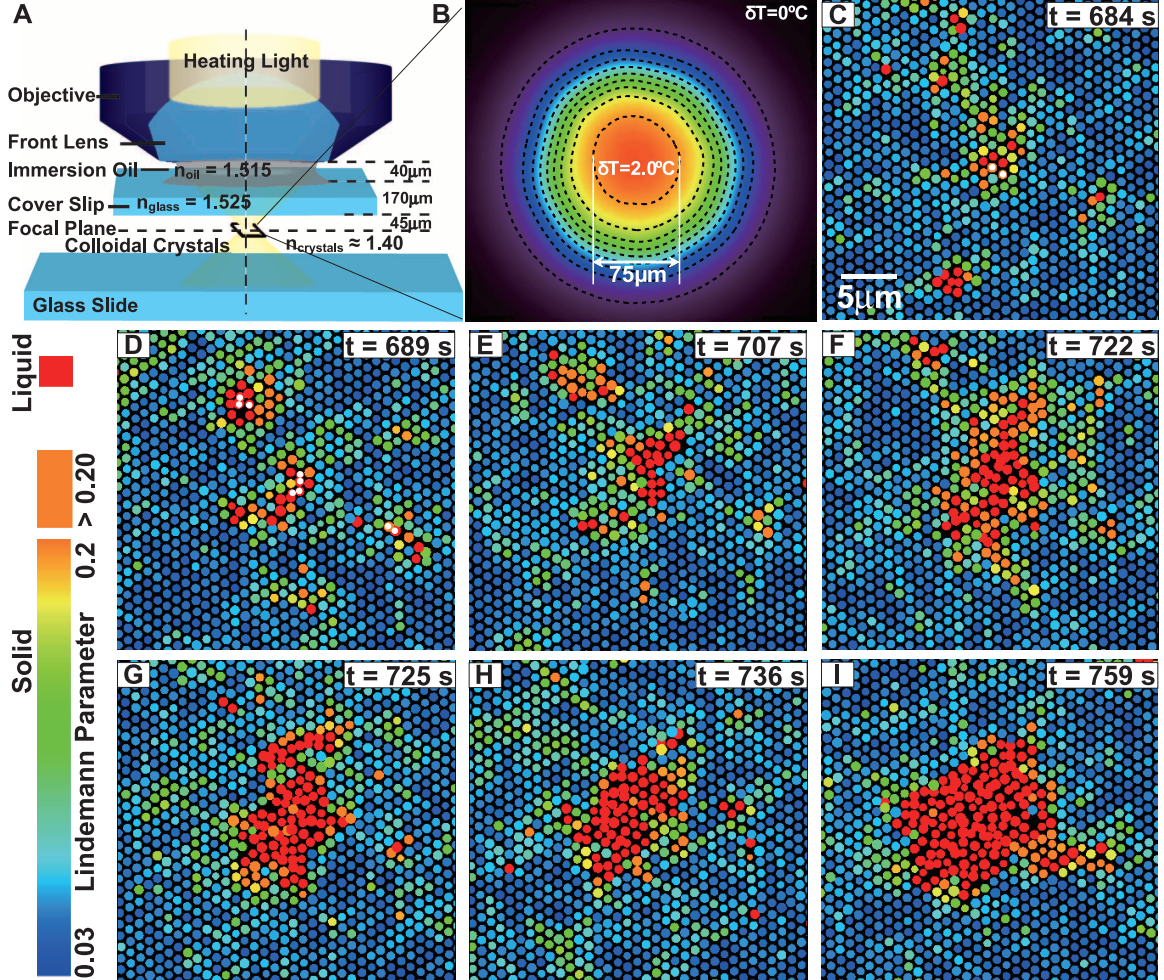
perature T_{amb} was controlled by the temperature controller on the microscope with a resolution of 0.1°C (a volume fraction of 0.4%), and $\delta T = 2.0^\circ\text{C}$ was the local optical heating effect, which was reached 2 s after the light was turned on (15). The crystal was superheated when $\Delta T \equiv T_{\text{amb}} + \delta T - T_m \equiv T - T_m > 0$ —that is, $-\Delta \phi \equiv \phi_{\text{amb}} - \delta \phi - \phi_m \equiv -(\phi_m - \phi) < 0$. Because the light was focused by the objective, the heating was the strongest in the focal plane and decayed to $0.98T$ at about ± 30 layers in the z direction (10) and was uniform in the center of the xy plane (Fig. 1B, temperature profile). Nucleation started randomly in the $\pi(38 \mu\text{m})^2$ by $(\pm 10 \mu\text{m})$ region of interest, hence the melting was homogeneous. To capture the initial stage of the nucleation, we rapidly scanned ± 10 layers along the z direction within 2 s every ~ 20 s and only recorded those nucleations that started near the focal plane. Nucleus volumes were measured from three-dimensional (3D) scans (movie S2) (10), while dynamics were measured by monitoring the largest 2D cross section of nucleus. The observation time scale ranged from 1 to 4000 s, which enabled us to explore both the strong superheating where melting was catastrophic and the weak superheating where nucleation was rare. The particle motions were recorded with a charge-coupled device (CCD) camera at 15 frames per second. Particle positions were tracked from the image analysis (17) in the crystal phase but not in the liquid phase, owing to their blurry images. Experimental details are given in (10).

In the theory of defect-mediated melting, defects are generated from a perfect crystal as temperature increases. They diffuse, coalesce, and form nuclei above the melting point. There is controversy, however, over which type of defect is the homogeneous melting precursor. In contrast to the conventionally suggested dislocations (18, 19), interstitials, and vacancies (20), recent simulations with different pair potentials showed that the precursors are collective loop motions when catastrophic melting at the superheating limit is avoided by using a slow heating rate (21, 22). In our colloidal samples, the heating rate was infinitely slow (the temperature was kept constant) so that the kinetics can be accurately measured. We also observed that the precursors were local particle exchanges instead of any obvious defects. As shown in Fig. 1, C and D, and movie S3 (10), particles marked by a white spot left their lattice sites, but the crystalline structure remained intact, which indicates that the particles swapped positions with their neighbors and formed closed loops in three dimensions. This is not surprising because it is the easiest way to move particles around in a perfect lattice. Moreover, we observed that local particle exchanges were surrounded by large-Lindemann-parameter

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Fig. 1. (A) The schematic of local optical heating. **(B)** The measured temperature profile in the focal plane under the light intensity used in most of the measurements, including Figs. 1, 2, and 3B. The contour spacing is 0.2°C . The temperature difference in the $\pi(38\ \mu\text{m})^2$ by $20\ \mu\text{m}$ region of interest is less than 0.2°C . **(C to I)** A typical nucleation process at $\phi = 52.0\%$ (i.e., $\Delta T = 0.6^\circ\text{C}$) whose size evolution is shown by curve 2 in Fig. 2A. The heating light was turned on at $t = 0\ \text{s}$. The colors represent different values of the Lindemann parameter. Liquid particles labeled in red are defined by the orientational order parameter $\psi = |\sum_{j=1}^n e^{6i\theta_j}|/nn < 0.6$, where θ_j is the orientation of the bond between the particle and its neighbor j , and nn is the number of nearest neighbors the particle has (15). **(C)** Two particles labeled in white spots left their lattice sites and swapped positions with their neighbors, but the lattice structure remained intact (movie S2) (10). **(D)** Three regions of particles in the midst of swapping positions surrounded by particles with large Lindemann parameter. **(E)** The regions with large Lindemann parameter particles coalesced



and formed a liquid nucleus. The nucleus reached the critical size in (F) and grew into a noncircular postcritical nucleus in (G), then became smaller and circular in (H) and rapidly expanded in (I).

(large- L) particles. The colors in Fig. 1, C to I, represent the values of the Lindemann parameter L , which reflects the motion amplitude of the particle relative to its equilibrium position (15). The large- L regions lasted for up to 1 min before they relaxed back to normal crystals or transformed into a liquid nucleus (Fig. 1E). Consequently, nucleation can be considered a two-step process: crystal $\rightarrow$ crystal with domains of large- L particles $\rightarrow$ nucleation. This is in accordance with Ostwald's step rule (23), that intermediate metastable or unstable states commonly exist in real nucleation processes to lower the free-energy barrier between the child and parent phases.

On the basis of the nucleation behaviors, we divide the superheating range into three regimes: weak superheating ($\Delta T \leq 0.5^\circ\text{C}$, i.e., $\phi \geq 52.5\%$, no coalescence between nuclei), intermediate superheating ($0.5^\circ\text{C} \leq \Delta T \leq 0.8^\circ\text{C}$), and strong superheating ($\Delta T \geq 0.8^\circ\text{C}$ —that is, $\phi \leq 51.0\%$, all nuclei irreversibly grow bigger). Figure 2A shows some typical nucleus-size evolutions under the weak, intermediate, and strong superheating regimes. A newly formed nucleus can be

large if it is formed in a large large- L region (Fig. 2A, jumps in curves 1 and 4). Such nuclei are smaller than the critical size under weak superheating. Under strong superheating, however, the critical nucleus is small (Fig. 2B), so that all the nuclei irreversibly grow bigger, and there are no obvious subcritical nuclei. Under the intermediate superheating regime, both types of nucleation paths (with and without the subcritical stage) were observed.

Under weak superheating, the nucleation kinetics basically followed the classical nucleation theory: Subcritical nuclei formed and disappeared during the incubation period (which typically spanned 30 min to more than 1 hour) until one of them reached the critical size. The nucleus size usually changed slowly so that we had enough time to scan in the z direction and measure the nucleus volume V and the effective radius $r = (3V/4\pi)^{1/3}$ shown in Fig. 2B. The critical nucleus size r^* was measured by adjusting T_{amb} so that a nucleus became as stable as possible with an equal probability of growing or shrinking (10). From Eq. 1, $r^*(\Delta\phi) \sim (\Delta\phi - d\phi)^{-1}$ where $d\phi$ is the

forbidden gap caused by the strain energy (10, 24). In this gap, the superheated crystal cannot transform into the lower-energy liquid phase because a kinetic path is not available (24). $r^*(\Delta\phi)$ in Fig. 2B can be accurately fitted for $d\phi < 0.002$. This upper bound of the gap is smaller than our temperature resolution and comparable with our estimated $d\phi \approx 0.0046$ for hard spheres (10). The small $d\phi$ is negligible, hence the critical nucleus $r^* \approx 2\gamma/(n\Delta\mu)$, the chemical potential difference $\Delta\mu \approx L_m\Delta\phi/(\phi_m - \phi_f)$, where L_m is the latent heat per particle usually taken as a constant (24) and the free energy barrier $\Delta G^* \approx 16\pi\gamma^3/[3(n\Delta\mu)^2]$ under weak superheating (10). These relations yield $r^* \approx 2(\phi_m - \phi_f)\gamma/(nL_m\Delta\phi)$. Our measured $r^* \propto 1/\Delta\phi$ in Fig. 2B, hence the surface tension γ is approximately a constant (10). If we take hard sphere's $L_m = 1.168 k_B T$ (25), where k_B is the Boltzmann constant, then $\gamma = 0.84 k_B T/\sigma^2$ and $\Delta\mu = 23.8 k_B T\Delta\phi$, which are comparable with those of hard sphere (26, 27). Comparing our fitted $r^* = 0.156\sigma(\phi_m/\Delta\phi)$ near ϕ_m from Fig. 2B and $r^* = 0.156\sigma(\phi_f/\Delta\phi)$ for the crystallization of hard spheres near ϕ_f (26), we found that melting and freezing have the same

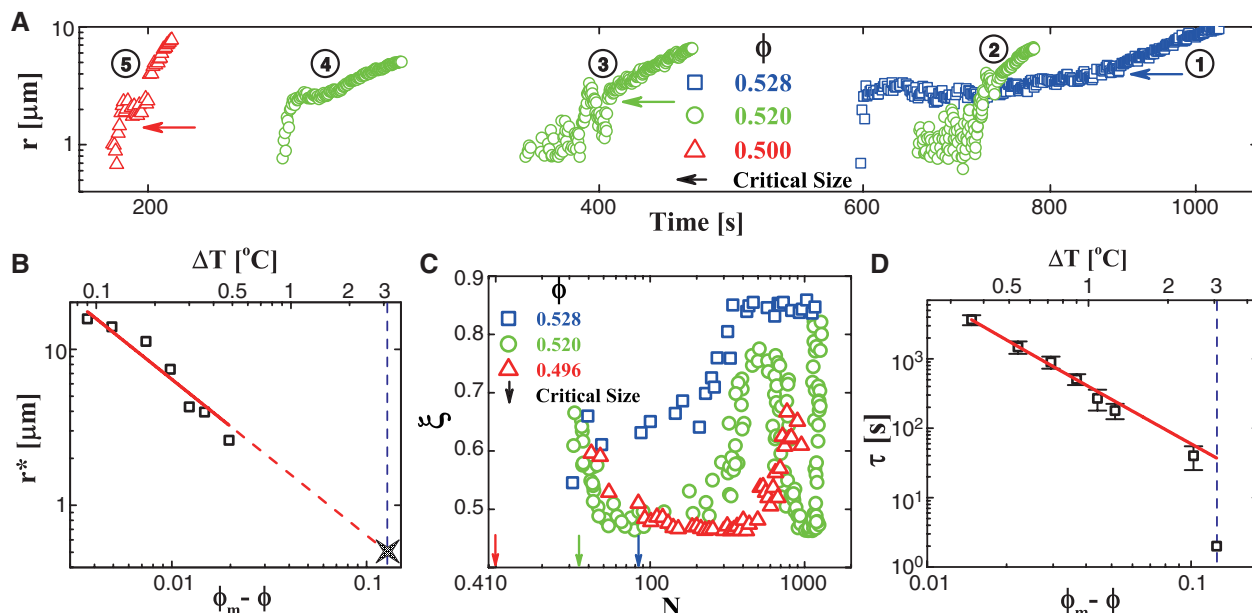


Fig. 2. (A) The evolution of the effective radius of nucleus at $\phi = 0.528$ ($\Delta T = 0.4^\circ\text{C}$, curve 1), 0.520 ($\Delta T = 0.6^\circ\text{C}$, curves 2, 3, and 4), and 0.500 ($\Delta T = 1.1^\circ\text{C}$, curve 5) (movie S4) (10). (B) The critical nucleus radius $r^*(\Delta\phi)$ (squares) is fitted by $(\Delta\phi)^{-1}$ (solid line) and extrapolated to the superheat limit. (C) The shape factor ξ as a function of the particle number in the cross section of the

nucleus for three nucleation processes at $\phi = 0.528$ (blue squares), 0.520 (green circles), and 0.496 (red triangles). The two minima of the $\phi = 0.520$ curve and the one minimum of the $\phi = 0.496$ curve correspond to nuclei coalescence. (D) The incubation time τ (squares symbols) is fitted by $(\Delta\phi)^{-2}$ (solid line). The vertical dashed lines in (B) and (D) denote the superheat limit.

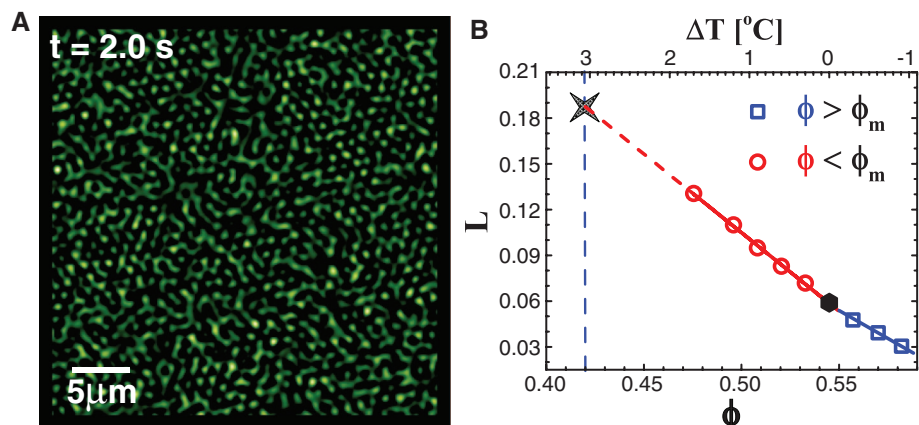


Fig. 3. (A) Melting started catastrophically from everywhere at $\phi = 41\%$. (B) The Lindemann parameter is extrapolated to the superheat limit $\phi_s = 42\%$.

prefactor in the colloidal system, although this result was only predicted in molecular systems (24).

Further complicating the nucleation kinetics, multiple large- L regions or liquid nuclei can coalesce especially under strong superheating (movies S3 and S6) (10). After coalescence, the nuclei became more nonspherical but eventually relaxed into spheres after a few minutes (fig. S4) (10). We characterized the shape of the nucleus by measuring the 2D shape factor $\xi = 4\pi A/l^2$, where A is the area and l is the perimeter of the nucleus. $\xi = 1$ for a circle and <1 for a noncircle. When a nucleus evolves without the influence of nearby nuclei or large- L regions, it is more noncircular at small sizes because the shape is more vulnerable

to thermal fluctuations (Fig. 2C, the $\phi = 0.528$ curve). Under strong superheating, nuclei often coalesce and become more noncircular in shape (Fig. 2C, the $\phi = 0.520$ and 0.496 curves). Noncircular nuclei tend to become smaller circular nuclei even when they are larger than the measured mean critical size (Figs. 1, G and H, and 2A, the oscillations near the critical size in curves 2 and 3). Hence, noncircular nuclei appear to have larger critical sizes than circular ones (28), which is in line with the observation of the nucleus shrinking after coalescence in crystallization (29) because coalescence often makes a nucleus more noncircular.

We measured the incubation time τ as the time taken for the first post-critical nucleus to

form (15). In our 90,000-particle region of interest, τ has large fluctuations (such as Fig. 2A, curves 2, 3 and 4). Hence, we averaged each τ in Fig. 2D over ~ 15 nucleation processes. The measured τ in Fig. 2D agree with the $\tau(\Delta\phi) \sim \Delta\phi^{-2}$ from the classical nucleation theory under weak superheating (9) but become smaller under stronger superheating. We attribute this departure to the more frequent coalescence of nuclei under stronger superheating, which can speed up the incubation process (movie S5) (10).

The superheat limit of most molecular crystals is $\sim 1.2T_m$ —crystals become unstable and catastrophically melt without incubation at $\sim 20\%$ above their melting point (6, 7, 30–32). However, the superheat limit of colloidal crystals is not known. Our colloidal crystals are metastable and melt via a nucleation mechanism, with a free energy barrier at $\phi = 43\%$, but become unstable and melt uniformly like a spinodal in 2 s at $\phi = 41\%$ (Fig. 3A and movie S6) (10), hence the superheat limit is at $\phi_s = 42 \pm 1\%$. This is 23% lower than $\phi_m = 54.5\%$, analogous to the supercool limit of hard spheres at the glass transition point $\phi_g^{HS} = 58\%$, which is 17% above $\phi_f^{HS} = 49.4\%$. In theory, the superheat limit may depend on a series of instability points of the crystalline state. These instability points are the kinetic instability (31), the Born criterion (32, 33), and the isochoric (meaning the liquid and the superheated solid have equal volume) (33), isentropic (equal entropy) (34), and the isenthalpic (equal enthalpy) (34) points. We found the superheat limit 42% is consistent with the measured Lindemann and Born instabilities (2, 31) but lower than the isochoric

$\phi = \phi_f^{HS} = 49.4\%$, which is the isentropic and the isenthalpic $\phi = 51\%$ (26) of hard spheres. At $\phi_s = 42\%$, the extrapolated Lindemann parameter $L_s = 0.187$ (Fig. 3B), which is close to $L = 0.176$ at the crystal-liquid interfaces and $L_s \cong 0.2$ for most molecular crystals (35). Moreover, the extrapolated $r_s^* = 0.59 a$ at $\phi_s = 42\%$, where a is the lattice constant (Fig. 2B). These results further confirm that $\phi_s = 42\%$ where the crystal is about to break down from the single-particle scale.

This experimental system enables us to investigate complex dynamics during the 3D homogeneous melting, including the nucleation precursor and nucleus evolution. It also provides a platform to study single-defect effects on melting and homogeneous nucleation in solid-solid transitions, which are difficult to be measured in molecular crystals.

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Supplementary Materials

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Materials and Methods

Supplementary Text

Figs. S1 to S4

References

Movies S1 to S6

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A Local Proton Source Enhances CO₂ Electroreduction to CO by a Molecular Fe Catalyst

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Electrochemical conversion of carbon dioxide (CO₂) to carbon monoxide (CO) is a potentially useful step in the desirable transformation of the greenhouse gas to fuels and commodity chemicals. We have found that modification of iron tetraphenylporphyrin through the introduction of phenolic groups in all ortho and ortho' positions of the phenyl groups considerably speeds up catalysis of this reaction by the electrogenerated iron(0) complex. The catalyst, which uses one of the most earth-abundant metals, manifests a CO faradaic yield above 90% through 50 million turnovers over 4 hours of electrolysis at low overpotential (0.465 volt), with no observed degradation. The basis for the enhanced activity appears to be the high local concentration of protons associated with the phenolic hydroxyl substituents.

The catalytic reductive transformation of carbon dioxide (CO₂) to fuels and commodity chemicals is one of the most important contemporary energy and environmental challenges. A highly negative potential is required to inject an electron into CO₂ (1, 2). Reaction pathways that would go through the intermediacy of the resulting anion radical are therefore quite unreasonable in terms of energy and activation. Focusing on the conversion to carbon monoxide (CO), a number of catalysts—mostly coordina-

tion complexes of low oxidation state transition metals—have been described (3–5). Among them, iron(0) porphyrins, electrochemically generated from the iron(II) porphyrin by two successive electron uptakes at a mercury or a glassy carbon electrode, are efficient, CO-selective, and durable catalysts provided they are coupled, in the framework of an electron-push-pull process, with Lewis acids (6) or weak Brønsted acids (7, 8). On the basis of this observed favorable role of proton donors, we reasoned that acid groups attached to the catalyst molecule should have a strong accelerating effect in view of the large local concentration of acid thus present, which would be impossible to introduce in such amounts in solution in the context of bimolecular reaction.

We have indeed found that modification of tetraphenylporphyrin (TPP) through the introduc-

tion of phenolic groups in all ortho and ortho' positions of the TPP phenyl groups, as shown in Fig. 1, leads to a considerable increase of catalytic activity. This is shown in Fig. 2, in which we plot the log of the turnover frequency (turnover number per unit of time), *TOF*, against the overpotential, η (difference between the standard potential of the CO₂/CO couple and the operating electrode potential). The variation of the *TOF* with the overpotential obtained from cyclic voltammetry of FeTDHPP in *N,N'*-dimethylformamide (DMF) + 2M H₂O in the presence of a saturating concentration of CO₂ (0.23 M) is shown as a thick gray segment.

For such molecular catalytic reactions, in which the catalyst is a well-defined molecule, with a well-defined standard potential, turnover frequency and overpotential were traditionally viewed as independent parameters, not allowing a precise comparison of catalyst performances. The obvious assertion that a good catalyst is characterized by a high *TOF* and a small η (and vice versa) is indeed not very helpful in this purpose. It has recently been shown (9) that turnover frequency and overpotential are in fact linked, for a given catalyst, by a relationship that may be formulated as in Eq. 1 (10) ($f = F/RT$, where F is Faraday's constant and R the gas constant):

$$\frac{1}{TOF} = \frac{1}{k_{cat}} + \frac{\exp[f(E - E_{cat}^0)]}{k_{cat}} + \frac{\sqrt{D} \exp[\frac{f}{2}(E - E_{cat}^0)]}{k_s \sqrt{k_{cat}}} \quad (1)$$

k_{cat} is the rate constant of the catalytic reaction, E_{cat}^0 the standard potential of the catalyst, k_s its

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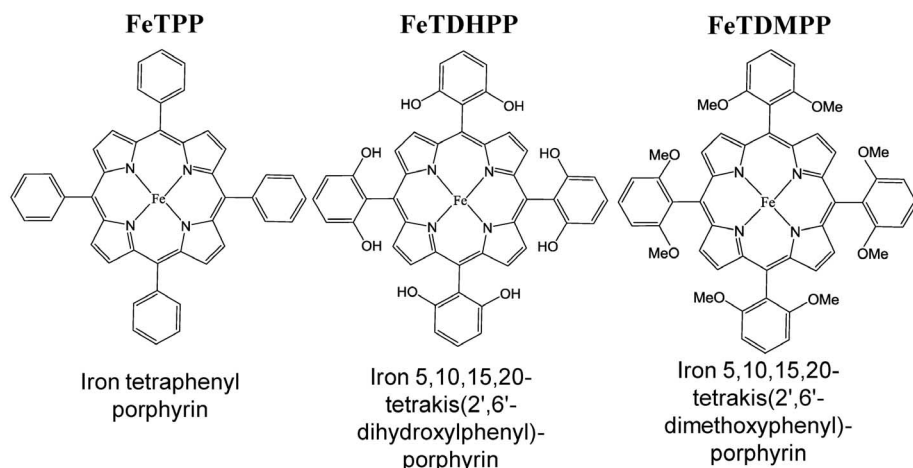


Fig. 1. Investigated iron porphyrins.

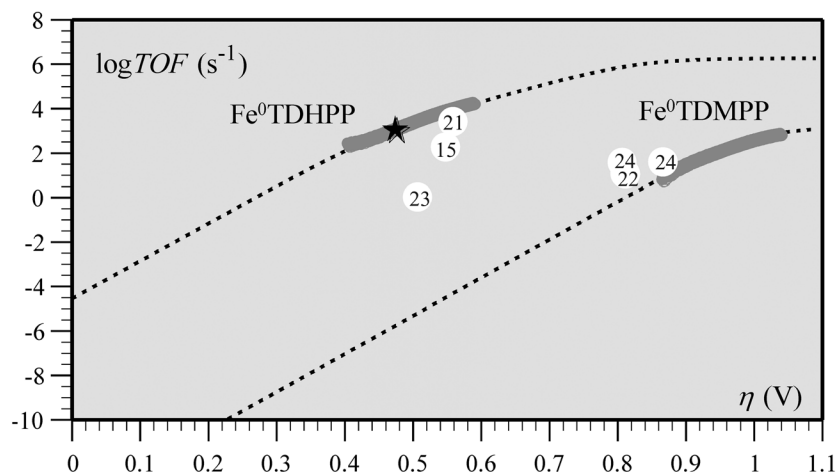


Fig. 2. Correlation between turnover frequency and overpotential for the series of CO₂-to-CO electroreduction catalysts listed in Table 1. Thick gray segments indicate *TOF* values derived from “foot-of-the-wave analysis” of the cyclic voltammetric catalytic responses of Fe⁰TDHPP and Fe⁰TDMPP in the presence of 2 M H₂O. Dashed lines indicate Tafel plots for Fe⁰TDHPP (top) and Fe⁰TDMPP (bottom). Also shown are *TOF* and η values from preparative-scale experiments: The star indicates Fe⁰TDHPP (this work), and circled numbers indicate the published references for other catalysts specified in Table 1.

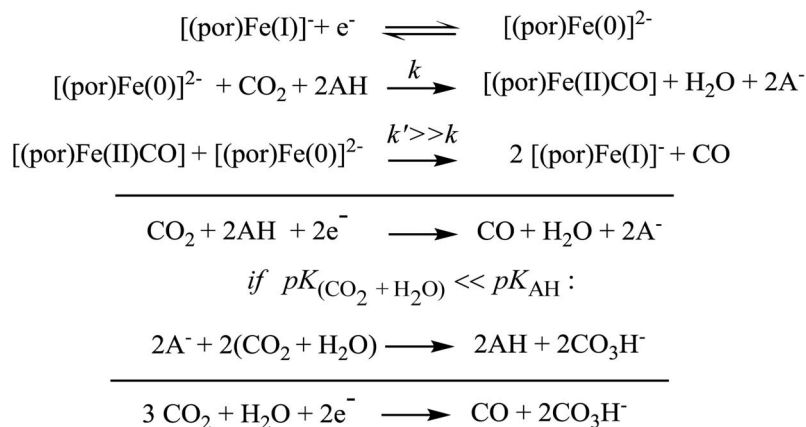


Fig. 3. Simplified reaction scheme for CO₂ reduction by iron(0) porphyrins.

standard rate constant, and D its diffusion coefficient. An alternative and equivalent formulation in Eq. 2 introduces the overpotential, η , defined earlier, and the turnover frequency at zero overpotential, TOF_0 (Eq. 3):

$$\frac{1}{TOF} = \frac{\exp[-f(E_A^0 - E_{\text{cat}}^0)]}{TOF_0} + \frac{\exp(-f\eta)}{TOF_0} + \frac{\sqrt{D} \exp(-\frac{f\eta}{2})}{k_s \sqrt{TOF_0}} \quad (2)$$

$$\eta = E_A^0 - E, TOF_0 = k_{\text{cat}} \exp[-f(E_A^0 - E_{\text{cat}}^0)] \quad (3)$$

(E_A^0 is the standard potential of the global reaction being catalyzed.)

This formulation characterizes the catalyst with three parameters: E_{cat}^0 , the standard potential of the catalyst redox couple, TOF_0 , a measure of intrinsic catalytic activity from a chemical standpoint, and $k_s/\sqrt{D}$, a measure of the conversion efficiency of the oxidized precatalyst to the reduced active catalyst, relative to mass transport. A graphical representation of Eq. 2 is given in Fig. 2 (dashed line), taking as an illustrative example the FeTDHPP catalyst. There are three successive overpotential domains. At large η , when the electrode potential is set well above the catalyst standard potential, the *TOF* is governed solely by the catalytic rate constant, regardless of the overpotential. In the opposing situation ($E \gg E_{\text{cat}}^0$), $\log TOF$ is a linearly increasing function of the overpotential with slope $f = f/\ln 10$ (1/59.3 mV at 25°C). In the transition between these two regimes, the system is controlled partly by the electron transfer and transport term, $k_s/\sqrt{D}$, giving rise to a linearly increasing function of the overpotential, with slope half the value in the preceding domain. With fast electron transfer catalysts, this intermediary zone tends to vanish.

One consequence of the existence of such a relationship is that one may select the electrolysis electrode potential with no necessity that it should be close to or more negative than the catalyst standard potential. As exemplified later on, it may indeed be more advantageous to operate at low overpotential, even though the resulting *TOF* decreases with overpotential.

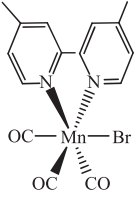
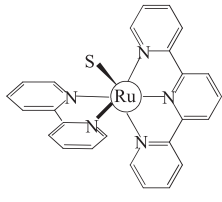
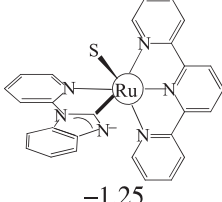
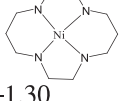
The $\log TOF$ versus η correlation diagram in Fig. 2 provides the basis for a rational comparison of the performances of the various molecular catalysts reported so far for the electroreduction of CO₂ to CO. Construction of the diagram requires an estimation of the standard potential of the CO₂/CO couple, $E_{\text{CO}_2/\text{CO}}^0$, in order to assign a value to the overpotential in each case. $E_{\text{CO}_2/\text{CO}}^0$ is known as a function of the pH when water is the solvent (11). Adaptation to DMF and acetonitrile, taking into account the stronger acid present, leads to the values given in the first column of Table 1, resulting from estimation that takes into account

the electron-, acid-, and CO₂-stoichiometry. Without going into the full mechanistic details, the catalytic reduction of CO₂ to CO encompasses the stoichiometric reactions depicted in Fig. 3. There are two cases: If the acid introduced the solution is stronger than (CO₂ + H₂O), $E_{\text{CO}_2/\text{CO}}^0$ depends on the pK_a (where K_a is the acid dissociation constant) of the acid present. In the opposite case, (CO₂ + H₂O) is the strongest acid in the medium. It thus neutralizes the conjugate base of the acid present, giving rise to a stoichiometry in which three molecules of CO₂ are involved in the two-electron reaction (Fig. 3). The way in which the values of $E_{\text{CO}_2/\text{CO}}^0$ reported for each case in Table 1 were obtained on these bases is detailed in the supplementary materials.

The star in Fig. 2 indicates the results of a preparative scale CO₂ electrolysis by using electrochemically generated Fe⁰TDHPP as the catalyst (fig. S1 and accompanying text). After 2 hours at a potential of −1.16 V versus normal hydrogen electrode (NHE), gas chromatographic analysis showed a 94% faradaic yield of CO, with 6% competing hydrogen formation; the averaged current density was 0.31 mA/cm² (fig. S5). This corresponds to logTOF = 3.5 at 0.466 V overpotential. The catalyst is also remarkably stable: Fifty million turnovers were achieved after 4 hours of electrolysis at this potential, with no degradation of the iron complex. The reason for selecting an electrolysis potential more positive than the standard potential of the catalyst couple derives from the subsequent considerations.

Rather than deriving the logTOF versus η relationship characterizing the Fe⁰TDHPP catalyst from a series of preparative scale electrolyses at various potentials, it can be obtained in a more rapid and detailed manner by treating the catalytic cyclic voltammetric responses of FeTDHPP as follows. In the absence of CO₂, Fe^{III}TDHPP shows three waves, in DMF, corresponding successively to the Fe^{III}/Fe^{II}/Fe^I/Fe⁰ redox couples (Fig. 4A). As with FeTPP (6–8), catalysis takes place at the most negative wave, meaning that the catalyst is the iron(0) complex. In the absence of CO₂, the Fe^I/Fe⁰ wave is not quite reversible at the slow scan rate, 0.1 V/s, at which the catalytic experiments are run. Raising the scan rate allows the determination of the Fe^I/Fe⁰ standard potential, $E_{\text{cat}}^0 = -1.333$ V versus NHE (fig. S3). Introduction of CO₂ results in a 60-fold increase of the current at the level of the Fe^I/Fe⁰ wave, (Fig. 4B) indicating a fast catalytic reaction. As discussed in detail elsewhere (9), the S-shaped current potential curve expected in these conditions is hampered by the occurrence of side phenomena such as substrate consumption, self-inhibition, deactivation of the catalyst, and possibly others that interfere more and more as the current increases. These undesirable phenomena are thus minimized at the foot of the current potential curve. Shown in Fig. 4, C and D, is how inspection of the foot of the catalytic current potential response can therefore be used to obtain the TOF as a function

Table 1. Catalysis of CO₂ reduction to CO, showing correlation between turnover frequency and overpotential for the series of catalysts listed.

Solvent $E_{\text{CO}_2/\text{CO}}^0$ V vs. NHE	Catalyst E_{cat}^0 (V vs. NHE)	η (V)	logTOF (s ^{−1})	logTOF ₀ (s ^{−1})	Ref
DMF + 2M H ₂ O −0.690	Fe ⁰ TDHPP −1.333	0.41–0.56	2.3–4.2	−4.6	This work (21)
	Fe ⁰ TDMPP −1.69	0.89–1.04	1.3–2.5	−13.9	
	Re(bipy)(CO) ₃ −1.25	0.57	3.3	−5.8	
DMF + HBF ₄ −0.260 *	{ <i>m</i> -(triphos) ₂ - [Pd(CH ₃ CN)] ₂ } −0.76	0.80	0.67	−7.5	(22)
CH ₃ CN + 5% H ₂ O −0.650	 −1.16	0.51	−0.05	−8.4	(23)
CH ₃ CN −0.650	 −1.30	0.87	1.5	−9.5	(24)
	 −1.25	0.81	1.5	−8.8	
1:4 H ₂ O CH ₃ CN −0.650	 −1.30	0.55	2.2	−7.1	(15)

*The large change in $E_{\text{CO}_2/\text{CO}}^0$ is due to the presence of a strong acid, HBF₄, which is much stronger than (CO₂ + H₂O) (supplementary materials).

of the electrode potential. The foot-of-the-wave analysis involves a portion of the current-potential curve that stands at potentials substantially more positive than E_{cat}^0 . It consists in plotting a fitting function *FIT* (supplementary text, section 6) against $1/\{1 + \exp[(f(E - E_{\text{cat}}^0))]\}$. The value of $2k[\text{CO}_2]$ is then derived from the slope of the linear portion of the *FIT* diagram, as shown in Fig. 4, D, F, and H. Using this value to obtain the logTOF-η relationship (Eqs. 1 to 3) closes the derivation of the catalyst characteristics from easy-to-measure cyclic voltammetric data as opposed to the more tedious preparative-scale tests.

The consistency of the two approaches is confirmed by the fact that the point representing the result of preparative-scale catalysis (Fig. 2, star) stands on the logTOF versus η curve derived from cyclic voltammetry. This also explains why the electrode potential for preparative scale electrolysis was selected to be more positive (by 170 mV) relative to the catalyst standard potential: In this potential region, the abovementioned side-phenomena, which would have perturbed the preparative-scale electrolysis as they perturbed the cyclic voltammetric responses, are minimized. It is in this low overpotential region that

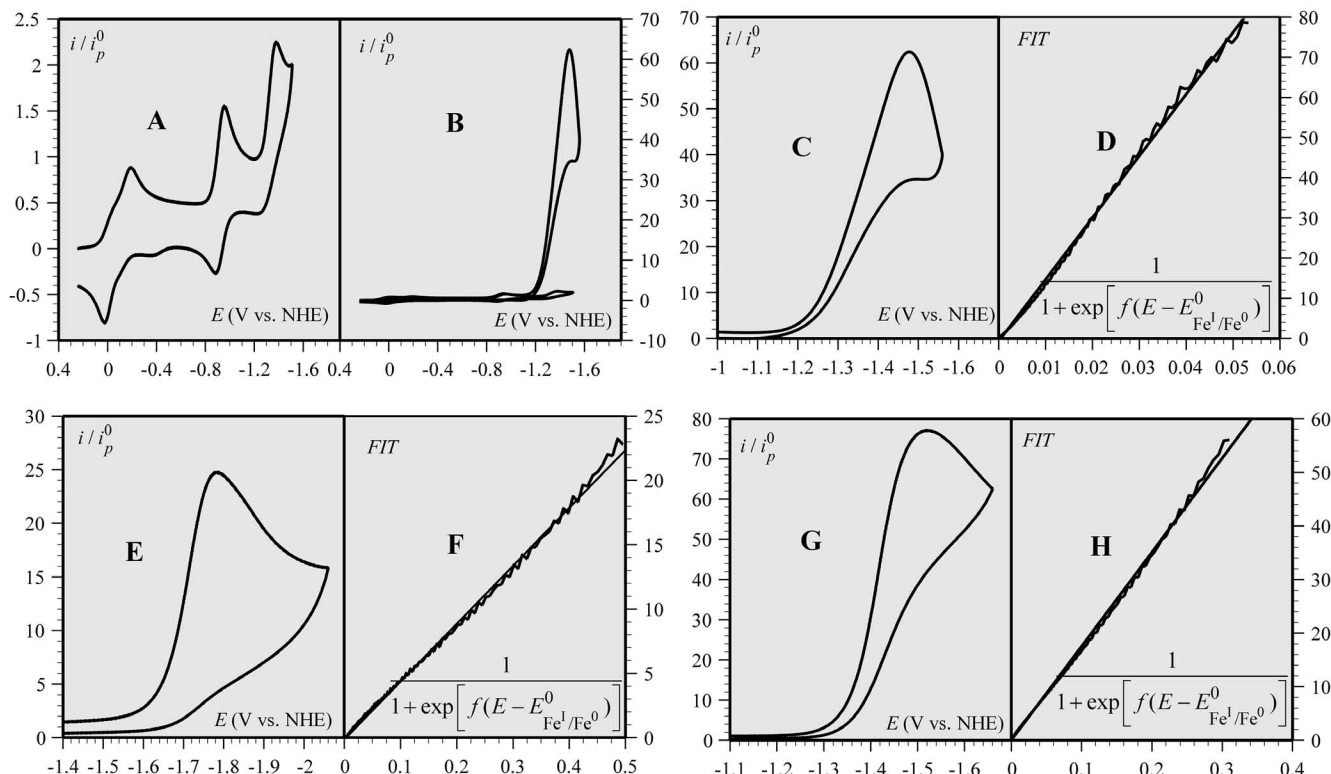


Fig. 4. Cyclic voltammetry in DMF + 0.1 M $n\text{-Bu}_4\text{NPF}_6$ electrolyte at 0.1 V/s of 1 mM of the three iron porphyrins (Fig. 1) after normalization to the $\text{Fe}^{\text{II}}/\text{Fe}^{\text{I}}$ peak current, i_p^0 . (A) FeTDHPP + 2 M H_2O . (B) FeTDHPP + 2 M H_2O in the presence (upper trace) and absence (lower trace) of 0.23 M CO_2 . (C) FeTDHPP

+ 2 M H_2O in the presence of 0.23 M CO_2 . (E) FeTDMPP + 2 M H_2O in the presence of 0.23 M CO_2 . (G) FeTPP + 3 M PhOH in the presence of 0.23 M CO_2 . (D, F, and H) Foot-of-the-wave analyses of the voltammograms in (C), (E), and (G), respectively.

the catalyst is used at the best of its catalytic capabilities: A current density of 0.31 mA/cm^2 at a 0.466 V overpotential is obtained even though the operating potential, -1.16 V versus NHE, is then well below the catalyst standard potential, -1.33 V versus NHE.

We may then compare, using the $\log \text{TOF}$ -versus- η representation of Fig. 2, the performances of the present $\text{Fe}^{\text{0}}\text{TDHPP}$ catalyst with those of the other molecular catalysts reported in the literature in DMF and CH_3CN as solvents. We excluded from the comparison a few systems for the following reasons. First, instable systems decompose after a few turnovers, as in (12) and (13). Then, the attractive system described in (14) likely involves heterogeneous interaction of a nickel cyclam complex with the mercury electrode surface [a comparison with a glassy carbon electrode is provided in (15)]. It is thus not possible to estimate the amount of catalyst participating in the reaction and hence to make a reliable comparison with the other catalysts in Table 1. Likewise, recent studies in which imidazolium (16) or pyridinium cation (17) are added to the aqueous solution and the electrode material (silver in the first case, platinum in the second) obviously plays a crucial, but still mysterious, role are clearly beyond the scope of molecular catalysis. The way in which the representative point of each catalyst was derived from the corresponding literature data are detailed in the supplementary text and in

Table 1, which gives the values of TOF_0 in each case. It appears that the $\text{Fe}^{\text{0}}\text{TDHPP}$ catalyst is slightly more efficient in terms of TOF (by a factor of ~ 10) than the most efficient catalyst previously reported. The latter is a rhenium complex, whereas the $\text{Fe}^{\text{0}}\text{TDHPP}$ catalyst uses a much cheaper metal.

Coming back to cyclic voltammetry, the catalytic properties of $\text{Fe}^{\text{0}}\text{TDMPP}$ were compared with those of $\text{Fe}^{\text{0}}\text{TDHPP}$ in order to highlight the essential role of the OH protons in the remarkable efficiency of the latter catalyst. The catalytic $\text{Fe}^{\text{0}}\text{TDMPP}$ wave (fig. S2 provides more cyclic voltammetry of $\text{Fe}^{\text{III}}\text{TDMPP}$ in the absence and presence of CO_2) and the associated foot-of-the-wave analysis, which underlies the lower dashed line in Fig. 2, are shown in Fig. 4, E and F. In the potential domain examined, this catalyst gives rise to rather high TOFs . However, this activity comes at the cost of large overpotentials. The comparison made at the level of intrinsic properties, as captured by TOF_0 , shows that $\text{Fe}^{\text{0}}\text{TDMPP}$ is a considerably poorer catalyst than $\text{Fe}^{\text{0}}\text{TDHPP}$ by a factor of ~ 1 billion, which is not even worth assaying at a preparative scale.

The comparison highlights the crucial role of the phenolic protons in this venture. This is confirmed by the observation that the $\text{TPPFe}(\text{I})/\text{Fe}(\text{0})\text{CO}_2$ catalytic wave increases with the addition of phenol in the solution (9). The exact mechanism of the interference of phenol is not known at present

but is likely to be of the same push-pull (electron-proton) type as previously proposed for other acids (8). Whatever the details of the mechanism, the enhanced $\text{Fe}^{\text{0}}\text{TDHPP}$ catalytic activity is thus related to the very high local concentration of phenolic protons. In this context, it is interesting to compare quantitatively the catalytic reactivities of $\text{Fe}^{\text{0}}\text{TDHPP}$ and of $\text{Fe}^{\text{0}}\text{TPP}$ in the presence of a high concentration of phenol. The catalytic response of $\text{Fe}^{\text{0}}\text{TPP}$ in the presence of 3 M phenol is shown in Fig. 4G, together with the corresponding foot-of-the-wave analysis in Fig. 4H. The corresponding characteristics of this catalyst are $E_{\text{cat}}^0 = -1.41 \text{ V}$ versus NHE and $k_{\text{cat}} = 2k[\text{CO}_2] = 3.2 \times 10^4 \text{ s}^{-1}$. The latter figure is to be compared with the rate constant for $\text{Fe}^{\text{0}}\text{TDHPP}$, $1.6 \times 10^6 \text{ s}^{-1}$, leading to an estimate that the eight phenolic OH groups in the molecule are equivalent to a 150 M phenol concentration. Similar reasons are likely to explain the high reactivities observed in the presence of a local proton donor in other cases, such as in the cleavage of O-O bonds, concerted with proton and electron transfer (18), in the catalytic conversion of O_2 into H_2O (19), and in the catalysis of hydrogen production and oxidation (20).

References and Notes

1. The standard potential of the $\text{CO}_2/\text{CO}_2^-$ couple has been estimated to be -1.97 V versus NHE in N,N' -dimethylformamide + 0.1M Et_4NClO_4 . The

- standard rate constant was 6×10^{-3} cm/s, with a transfer coefficient of 0.4 (2).
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 - The last term in Eq. 1 is lacking in the derivations of (9) because electron transfer between the electrode and the catalyst was assumed to be unconditionally fast. In the present case, catalysis is so fast that the catalyst electron transfer kinetics cannot be neglected. Full proof of Eq. 1 is given in the supplementary materials, section 5.
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Supplementary Materials

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Materials and Methods
Supplementary Text
Figs. S1 to S6
References (25–40)

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Dynamics of DNA Supercoils

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DNA in cells exhibits a supercoiled state in which the double helix is additionally twisted to form extended intertwined loops called plectonemes. Although supercoiling is vital to many cellular processes, its dynamics remain elusive. In this work, we directly visualize the dynamics of individual plectonemes. We observe that multiple plectonemes can be present and that their number depends on applied stretching force and ionic strength. Plectonemes moved along DNA by diffusion or, unexpectedly, by a fast hopping process that facilitated very rapid (<20 milliseconds) long-range plectoneme displacement by nucleating a new plectoneme at a distant position. These observations directly reveal the dynamics of plectonemes and identify a mode of movement that allows long-distance reorganization of the conformation of the genome on a millisecond time scale.

Supercoiling and changes in the supercoiling state are ubiquitous in cellular DNA and affect virtually all genomic processes (1). Proteins moving along the helical path of the DNA, for example, generate torsional stress, which produces twist (the over- or underwinding of the DNA double helix around its axis) and writhe (the coiling of the duplex axis around itself). Supercoiling affects the cell because it alters the conformation of the genome on two basic levels. First, supercoiling can induce local changes in the DNA structure, such as a locally destabilized or deformed duplex, which subsequently affect transcription or trigger protein binding (2–4). Second, supercoiling can induce global changes in the conformation of the genome, which bring distant DNA sequences together, thereby facilitating DNA compaction and site-specific recombination (5, 6). Genomic DNA is organized in topological domains of 10 to 100 kilobases (kb) that isolate topological changes from neighboring regions (1). Within these regions, torsion can rapidly transmit, allowing for long-range communication between distant genomic locations (7). To understand the cellular processes that are

affected by supercoiling, it is essential to comprehend its dynamics, of which virtually nothing is known. Do plectonemes move along DNA, and if so, by which process and on what time scale? We address these questions at the single-molecule level.

The dynamics of supercoiled DNA have been difficult to address experimentally. Static images obtained by electron and atomic force microscopy showed that supercoiled plasmids are plectonemic (8, 9). These images could not capture the dynamics, as DNA needs to be strongly immobilized for imaging. Measurements of site-specific recombination have provided indirect information on the speed of juxtaposition of DNA positions, but these results did not agree with theoretical predictions, leaving many unanswered questions about the dynamics and diffusion speed of plectonemes (6, 10). Single-molecule magnetic tweezers have proven to be an ideal platform to study DNA mechanics, as they can twist and apply a stretching force to individual DNA molecules (11). However, in the traditional implementation, this technique is limited, as it only measures the DNA end-to-end distance and not the position of a plectoneme along the DNA tether.

To visualize the dynamics of plectonemes directly along a single DNA molecule, we developed a magnetic tweezers apparatus (Fig. 1A) that pulls a fluorescently labeled DNA molecule sideways and visualizes it along its length using epi-fluorescence. Each experiment started

by creating plectonemes in a DNA molecule that was torsionally constrained between the flow cell surface and a magnetic bead by coiling it with a pair of magnets positioned above the DNA tether. The DNA molecule was positively supercoiled to a degree at which 25% of the DNA contour length was put into a plectonemic state (Fig. 1B and table S1). Subsequently, we brought an additional magnet near the side of the flow cell and removed the top magnets, thus pulling the DNA tether sideways at modest stretching forces (0.4 to 3.2 pN), which are readily generated by, for example, polymerases in vivo (12). We took all measurements on 21-kb DNA molecules, which are similar in length to the topological domains observed in genomic DNA (1). We covalently labeled the DNA molecules with Cy3 dyes attached by a long (~3-nm) linker at random positions along their length, resulting in a low labeling density of ~1/25 base pairs. An efficient oxygen-scavenging system (13) allowed us to monitor supercoiled DNA molecules for several seconds before photo-induced nicking occurred, which released all torsional stress. Cy3 labeling did not affect the mechanical properties of the DNA, as shown by the rotation curves of labeled molecules, which overlap with those of unlabeled molecules (Fig. 1B and fig. S1) (14).

We observed individual plectonemes in supercoiled DNA molecules in images acquired with 20-ms time resolution. As illustrated in Fig. 1C and movie S1 (14), plectonemes appear in the images as bright spots along the DNA, as the local DNA density is higher in the plectonemes. These bright spots were near-diffraction-limited, with a typical spot size of ~500 nm. The spots were not present in nonsupercoiled DNA and disappeared instantly if a DNA molecule was nicked (fig. S2) (14).

We observed that multiple plectonemes were present that appeared and disappeared and moved along the DNA. We analyzed the dynamics of plectonemes by converting the time series of images to kymographs, which plot the intensity profile along the DNA position versus time (Fig. 1D). Within the 2-s time scale of the kymograph in Fig. 1D, we observed many events in which plectonemes nucleated, moved, and disappeared

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some time later. We developed a fitting routine to count and extract the plectonemes' positions over time (figs. S3 to S5) (14). A typical result is shown in Fig. 1E, where individual plectonemes are marked by different colors.

We find that the number of plectonemes in the DNA varies substantially with ionic strength and applied force. Experiments were performed for a range of ionic strengths (concentration of NaCl [NaCl] = 20 to 300 mM) and forces (f = 0.4 to 3.2 pN) (Fig. 2A and movie S2) (14). We found that plectonemes are very dynamic at low forces and quickly move between positions along

the DNA. At higher forces, the dynamics become restricted, and single plectonemes remain at the same position for long periods of time (several seconds), giving rise to nearly static high-intensity bands in the kymographs. The number of plectonemes present at any given time varied from a single plectoneme for high-force and -salt conditions to about three for low-force and -salt conditions (Fig. 2, B and C). The mean size of individual plectonemes varied between ~1.7 and ~5.3 kb, which is consistent with the ~2.3 kb observed by electron microscopy (8) and also with predictions of Monte Carlo simulations (15).

Our results confirm the general trends described in two recent theoretical studies by Emanuel *et al.* (16) and Marko and Neukirch (17), which predict the presence of multiple plectonemes for low-force (<0.5 pN) or low-salt concentrations (<50 mM). At higher forces and increased salt concentrations (≥ 1 pN, >50 mM), Marko and Neukirch (17) predict the presence of only a single plectonemic domain in a 10-kb DNA molecule, whereas we observed that a few plectonemes could still be present under these conditions.

The number of plectonemes present in a DNA molecule will be set by the free-energy balance

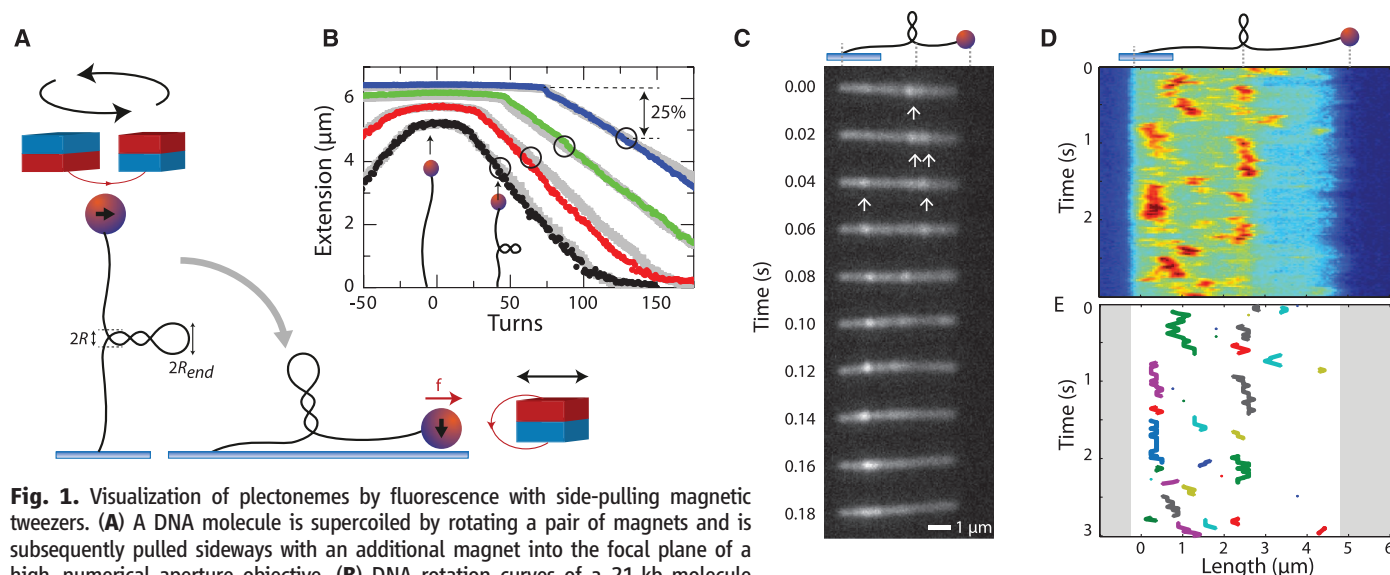
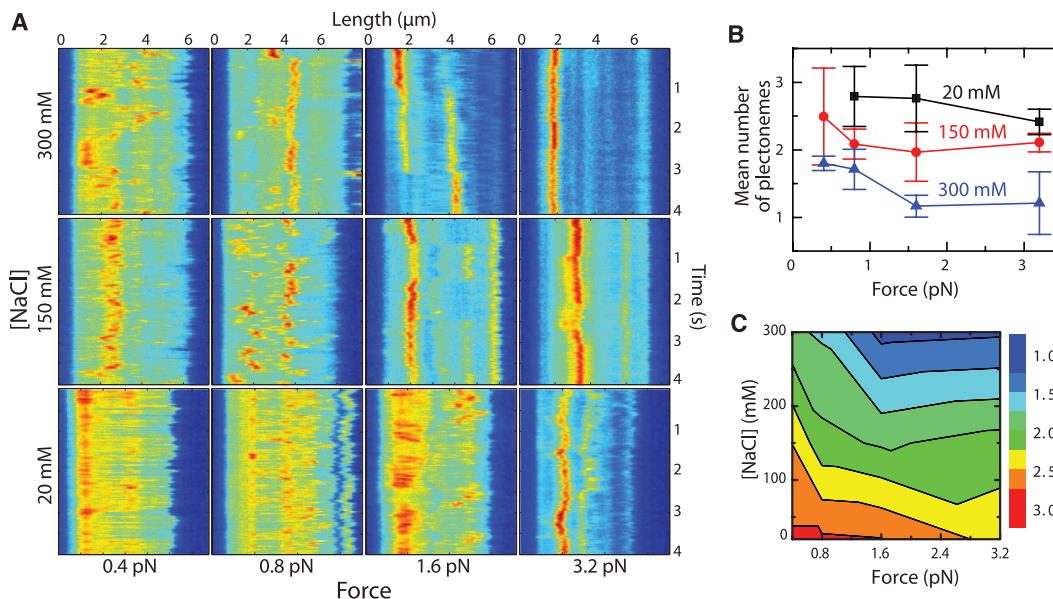


Fig. 2. The number of plectonemes and their dynamics depend on the applied stretching force and ionic strength. (A) Kymographs of DNA molecules for 12 different stretching force/ionic strength combinations (also available as movie S2). (B) The mean number of plectonemes increases with decreasing ionic strength ($n \geq 4$ DNA molecules per data point, error bars indicate SD). (C) Phase diagram showing the dependence of the number of plectonemes on applied stretching force and ionic strength.



between the change in enthalpy and the change in entropy upon the formation of an additional plectoneme (15). Entropy will favor the presence of multiple plectonemes, as they can occupy multiple positions along the molecule and distribute the writhe between them (16, 17). The energy cost required to bend the DNA in the plectoneme, however, will favor a single plectoneme. The structure of a plectoneme can be simplified to consist of an intertwined section and an end loop (Fig. 1A). The formation of an end loop with size $2R_{\text{end}}$ (where R_{end} is the end-loop radius) is energetically more costly than extending the intertwined region, making it unfavorable to form multiple plectonemes (18). Surprisingly, the data showed that multiple plectonemes were present in DNA molecules.

The observed number of plectonemes decreased for higher ionic strengths (Fig. 2, B and C), which can be understood by considering the plectoneme structure. Increasing ionic strength will screen the electrostatic repulsion between the highly charged DNA backbones, leading to a reduction of the plectoneme radius (R) compared with R_{end} , which is set by the mechanical bending of the DNA (19, 20). At high ionic strength, $R < R_{\text{end}}$, favoring a single plectoneme, but at low ionic strength, R increases and becomes approximately equal to R_{end} , reducing the free-energy penalty for forming additional plectonemes. This response was experimentally observed as the number of plectonemes increased with decreasing ionic strength (Fig. 2, B and C). The applied stretching force did not have a strong influence on the number of plectonemes (Fig. 2B). In contrast to ionic strength, an applied stretching force would reduce both R and R_{end} , making the free-energy penalty for the formation of an additional plectoneme rather insensitive to force.

We now turn to the dynamics of plectonemes. Unexpectedly, we observed two different types of motion: (i) diffusive motion, in which a plectoneme randomly moved along a DNA molecule (Fig. 3, A to C), and (ii) hopping, in which a plectoneme suddenly shrank or disappeared while simultaneously a new plectoneme nucleated at a different location (Fig. 4, A to C). First, we focus on diffusion. To quantify the diffusive behavior of plectonemes, we tracked the position of individual plectonemes with an extended lifetime (≥ 0.3 s) (Fig. 3B and fig. S6) (14). An example of the diffusional tracks for 24 plectonemes is shown in Fig. 3D. To extract the plectoneme diffusion constant D , we calculated the mean squared displacement (MSD) $\langle \Delta x^2(t) \rangle$, where t is time, Δx is the plectoneme displacement along the DNA, and $\langle \rangle$ denotes the time average (supplementary text S1 and S2) (14). As shown in Fig. 3E, we observe a near-linear relation of the MSD with time, $\langle \Delta x^2(t) \rangle = 2Dt$, which is characteristic for Brownian motion, indicating that plectonemes indeed move along DNA by one-dimensional diffusion, similar to the diffusion of knots in DNA (21).

Diffusion of a plectoneme not only requires the sideways motion of the plectoneme, but also the slithering motion of the DNA within the su-

percoil (Fig. 3C) (22). Notably, the diffusional constants that we experimentally observed were substantially lower than those predicted for the hydrodynamic drag of plectonemes (Fig. 3F and supplementary text S3) (14) and showed a rapid decrease with applied stretching force (Fig. 3, E and F). Surface effects caused by the proximity of the DNA molecule to the surface cannot explain such a large reduction in diffusion speed, as they result only in a very small increase in the viscous drag (supplementary text S4) (14).

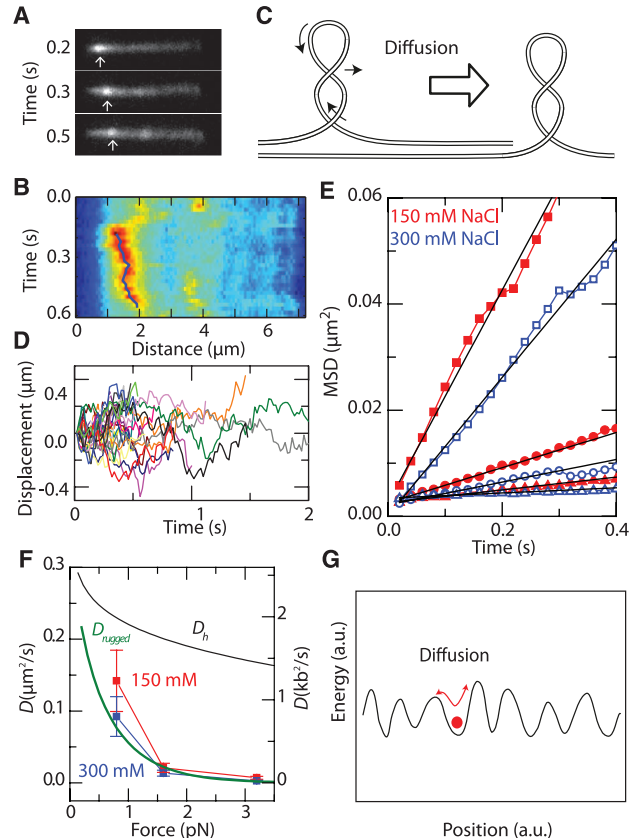
The unexpectedly slow diffusion of plectonemes can originate from different microscopic causes, which, in their most general form, may be represented by the presence of a rugged energy landscape that the plectoneme must navigate while moving along the DNA molecule. This energy landscape can derive from sequence-dependent mechanical properties, such as the intrinsic curvature and bendability of DNA, that create local energy barriers for diffusion (23). We observed that plectonemes are not altogether randomly distributed along a DNA molecule, but they have some preference for certain positions along the DNA (Fig. 2A).

We can quantitatively estimate the effect of a rugged energy potential on the diffusive motion of a plectoneme by expressing D as the product of the hydrodynamic diffusion constant D_h and a retardation factor $F(\epsilon)$. If we assume that the fluctuating part of the potential energy landscape along the DNA molecule obeys a Gaussian dis-

tribution, then $F(\epsilon) = \exp[-(\epsilon/k_B T)^2]$, where ϵ denotes the root mean square fluctuations in the energy potential, k_B is Boltzmann's constant, and T is temperature (24). As the bending energy for looping DNA scales as the square root of the applied stretching force (25), we propose a rugged-energy model with $\epsilon(f) \propto \sqrt{f}$, which provides an excellent fit (green line in Fig. 3F) to the experimental data compared with the simple hydrodynamic model (black line in Fig. 3F; see also fig. S7) (14). We found values for ϵ of $1k_B T$ (at 150 mM and 0.8 pN) to $2k_B T$ (at 300 mM and 3.2 pN), representing only a small modulation compared with the bending energies of the end loop, which are $14k_B T$ and $28k_B T$, respectively (25). Importantly, the values of ϵ are close to the thermal energy, allowing for diffusion to occur, albeit at a reduced speed.

The second mechanism by which plectonemes move along DNA is hopping, where one plectoneme suddenly shrinks or vanishes and a new one simultaneously nucleates at a different position along the DNA (Fig. 4, A to C). These hopping events were generally fast, occurring abruptly within the 20 ms of our single-frame time resolution, and spanned large distances that could not be explained by a diffusion mechanism (Fig. 4, A and B, and supplementary text S5) (14). The total length of DNA in plectonemes was constant at 25% in our experiments, so shrinking of a plectoneme necessarily resulted in the

Fig. 3. Plectoneme diffusion along a DNA molecule. (A) Images and (B) kymograph of a plectoneme diffusing along a DNA molecule (150 mM NaCl, 0.8 pN). Solid line in (B) denotes the diffusional track of the plectoneme as obtained by a fit to the intensity in the kymograph (fig. S6). (C) Diffusion of a plectoneme involves the sideways movement of the DNA but also a slithering motion within the plectoneme. (D) Example of diffusion traces. (E) MSD of plectoneme positions at 0.8 pN (squares), 1.6 pN (circles), and 3.2 pN (triangles) of stretching force. Lines are linear fits to the MSD (supplementary text S1). Solid symbols, 150 mM NaCl; open symbols, 300 mM NaCl. (F) Diffusion constants, corrected for the DNA extension at the experimentally probed forces (supplementary text S1), obtained from linear fits of (E). The observed diffusion constants are much lower than those predicted from a hydrodynamic diffusion model (black line) (supplementary text S3). A model incorporating a rugged energy landscape (green line) (see also fig. S7) fits the 300-mM data well. (G) Schematic diagram of plectoneme diffusion in a rugged energy landscape along a DNA molecule. a.u., arbitrary units.



growing or nucleation of a new plectoneme, and the observed nucleation rate (0.3 to 22 s^{-1}) is equivalent to the hopping rate. This rate decreased with increasing force and ionic strength (18) (Fig. 4D and supplementary text S6) (14), whereas the mean plectoneme lifetime (0.1 to 10 s) increased strongly with force and ionic strength (Fig. 4E). The distribution of plectoneme lifetimes showed a strong decrease with time (Fig. 4F); that is, most plectonemes are short-lived ($<0.1\text{ s}$).

We can explain the distribution of lifetimes by considering what happens after nucleation of a plectoneme. The plectoneme can either grow by absorbing more writhe or shrink by releasing writhe. The growing and shrinking of a plectoneme can be described by a random walk, and the lifetime of the plectoneme is then set by the first return to the origin of the walk—that is, the nucleation point. The probability of this first return at time t is given by $P(t) = \binom{t}{t/2} \frac{1}{t^{3/2}}$,

where $\binom{t}{t/2}$ denotes the binomial coefficient. This random-walk model described the experimental data well (blue lines in Fig. 4F; see also supplementary text S7) (14). We observed a similar scaling of the lifetimes for all experimental conditions (fig. S8) (14).

Hopping allows plectonemes to move over large distances. We observed maximum hopping distances of up to 15 kb ($\sim 5\text{ }\mu\text{m}$) in our 21-kb DNA molecules (fig. S9) (14). Interestingly, hop-

ping can occur over these large distances regardless of the rugged energy landscape in between, as the nucleation at the new location is independent of any energy barriers associated with the DNA between the shrinking plectoneme and the nucleation spot (Fig. 4G). Hopping is merely restricted by the energy required for nucleation of a new plectoneme and the subsequent transfer of writhe to the new plectoneme by the rotation of the intermediate DNA. The nucleation barrier depends on both applied stretching force and ionic strength, predicting lower nucleation rates at high force and high ionic strength (18), matching our observations (Fig. 4D).

A theoretical estimate of the viscous drag associated with the rotation and movement of the intermediate DNA during hopping (Fig. 4C) shows that it only grows slowly with distance and is much smaller than the drag for the diffusion of a plectoneme. Surprisingly, thermal fluctuations are expected to result in a hopping distance that scales linearly with time, in contrast to the distance for diffusive motion, which scales as the square root of time (supplementary text S8) (14). The lower drag allowed plectonemes to relocate by hopping over many kilobases in a fraction of a second, which would be impossible for diffusion of a plectoneme along the DNA molecule.

The observed dynamics of DNA supercoils reveal how plectonemes change the DNA conformation. We found that multiple plectonemes are present in a supercoiled DNA molecule un-

der applied force, with a typical density of one plectoneme per 10 kb . Diffusion of plectonemes was strongly dependent on applied stretching force, suggesting that it is hindered by local inhomogeneities in the DNA mechanical properties. In contrast, hopping of plectonemes results in a fast, long-range rearrangement of the DNA conformation, which may explain the fast search times for site juxtaposition of two distant DNA regions. Hopping can also aid to recruit a plectoneme to a DNA sequence that exhibits inherent curvature or to a site of protein-induced DNA bending. Such a mechanism will allow for changes in the conformation of the genome at the millisecond timescale, thereby triggering protein binding or influencing gene expression.

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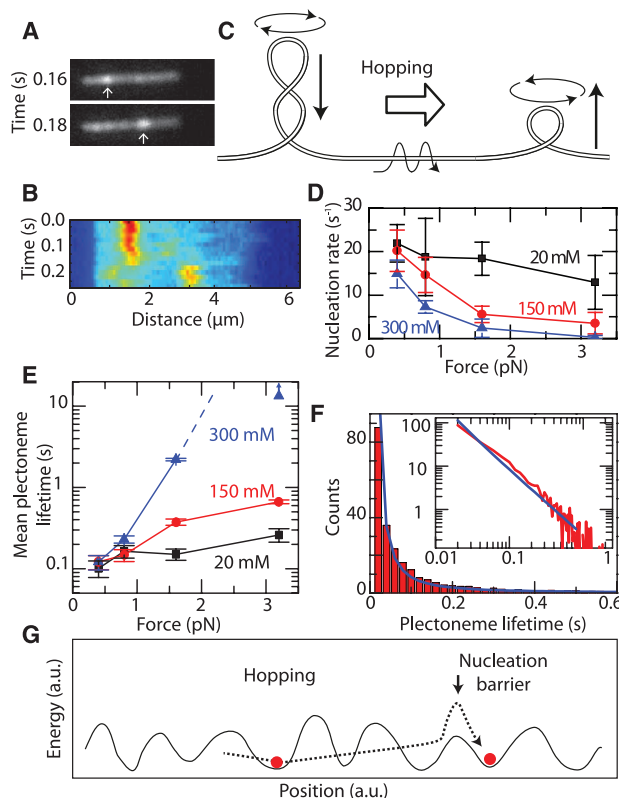
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Supplementary Materials

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Fig. 4. Plectoneme hopping along a DNA molecule. (A) Images and (B) kymograph of a hopping event (150 mM NaCl , 0.8 pN). (C) Hopping of a plectoneme involves the nucleation of a new loop at a different location and the transfer of writhe by rotation and sideways motion of the intermediate DNA. (D) Nucleation rate of plectonemes as a function of force at different ionic strengths (supplementary text S6). $n \geq 4$ DNA molecules for each condition; error bars indicate SD. (E) Mean plectoneme lifetime versus force. $n \geq 4$ DNA molecules for each condition; error bars indicate SD. The data point at 3.2 pN , 300 mM NaCl only provides a lower bound, as nicking of the DNA molecule limited the observed lifetimes. (F) Histogram of the plectoneme lifetimes at 150 mM NaCl , 0.8 pN . (Inset) The same data shown on a log-log scale. A model for the first return to the origin of a random walk for the same number of events agrees well with the experimental data (blue lines). (G) Schematic diagram showing that hopping can bypass roughness in the energy landscape that impedes diffusion along the molecule.



Complex Dental Structure and Wear Biomechanics in Hadrosaurid Dinosaurs

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Mammalian grinding dentitions are composed of four major tissues that wear differentially, creating coarse surfaces for pulverizing tough plants and liberating nutrients. Although such dentition evolved repeatedly in mammals (such as horses, bison, and elephants), a similar innovation occurred much earlier (~85 million years ago) within the duck-billed dinosaur group Hadrosauridae, fueling their 35-million-year occupation of Laurasian megaherbivorous niches. How this complexity was achieved is unknown, as reptilian teeth are generally two-tissue structures presumably lacking biomechanical attributes for grinding. Here we show that hadrosaurids broke from the primitive reptilian archetype and evolved a six-tissue dental composition that is among the most sophisticated known. Three-dimensional wear models incorporating fossilized wear properties reveal how these tissues interacted for grinding and ecological specialization.

Hadrosaurids were the dominant large herbivores of Late Cretaceous Europe, Asia, and North America (1). Gut and fecal contents show that these gregarious, facultative bipeds (Fig. 1A) with broad ducklike bills grazed on horsetail, fern, and primitive angiosperm ground cover and browsed on conifers (2). These tough plants, laden with siliceous phytoliths and/or exogenous grit that scoured their teeth (1), were pulverized using dentitions consisting of columns of developing and functional teeth with flattened horse- and bison-like grinding surfaces (3, 4) (Fig. 1, B to D). It's likely that exploitation of these resources, which were presumably not as accessible to their forebears who had shearing teeth (5–7) (Fig. 1E), facilitated the extensive hadrosaurid radiation (8, 9).

The traditional model of hadrosaurid chewing surfaces includes only the primitive reptilian (Sauria) dental tissues enamel (hard hypermineralized material) and orthodentine (“dentine”; soft bonelike tissue) (3, 4) (Fig. 2A). Accordingly, file-like crests and basins would have formed on tooth surfaces because of differential wear resistance as the teeth moved across the chewing surface (6) (Fig. 1C).

This model contrasts with what we see in more complex mammalian grinding teeth (10–14) (Fig. 1D). In mammals, the major tissues, besides enamel and orthodentine, include soft secondary dentine (that forms a lower tier within basins, sealing

the pulp cavity to prevent abscesses) and coronal cementum (a derived root attachment tissue that migrates onto the crowns, reducing stress on the brittle crests by transmitting loads among tissues).

The current model of hadrosaurid dental architecture is simplistic, lacking both crest-supporting

and abscess-preventative tissues. Furthermore, enamel is present only in the leading teeth in the lower batteries (Fig. 2A), thereby leaving no hard tissues to form subsequent crests (Fig. 2B). Finally, conspicuous features are unaccounted for, including granular material between teeth and filling pits within basins, slicing planes on leading-edge teeth, and branched and linear ridges within the basins (15) (Fig. 2, B and C).

How was mammal-like grinding achieved in hadrosaurids? We provide answers by (i) characterizing tissue compositions and chewing surface morphologies in hadrosaurids and outgroups, (ii) mechanically testing the tissues for wear-relevant attributes, (iii) using those properties in a tribological (study of wear) model to reveal the biomechanics of grinding-surface formation, and (iv) summarizing these findings in an evolutionary context.

Comprehensive phylogenies for the Hadrosauridae (8) (fig. S1) and Ornithischia (16) were used to identify 27 taxa representing dental variation throughout the ornithopodan radiation (table S1). The tissue compositions were determined from intact batteries and transversely sectioned teeth using dissecting and/or polarizing microscopy (17). Casts of worn chewing surfaces were made and the morphologies digitally captured using laser scanning confocal

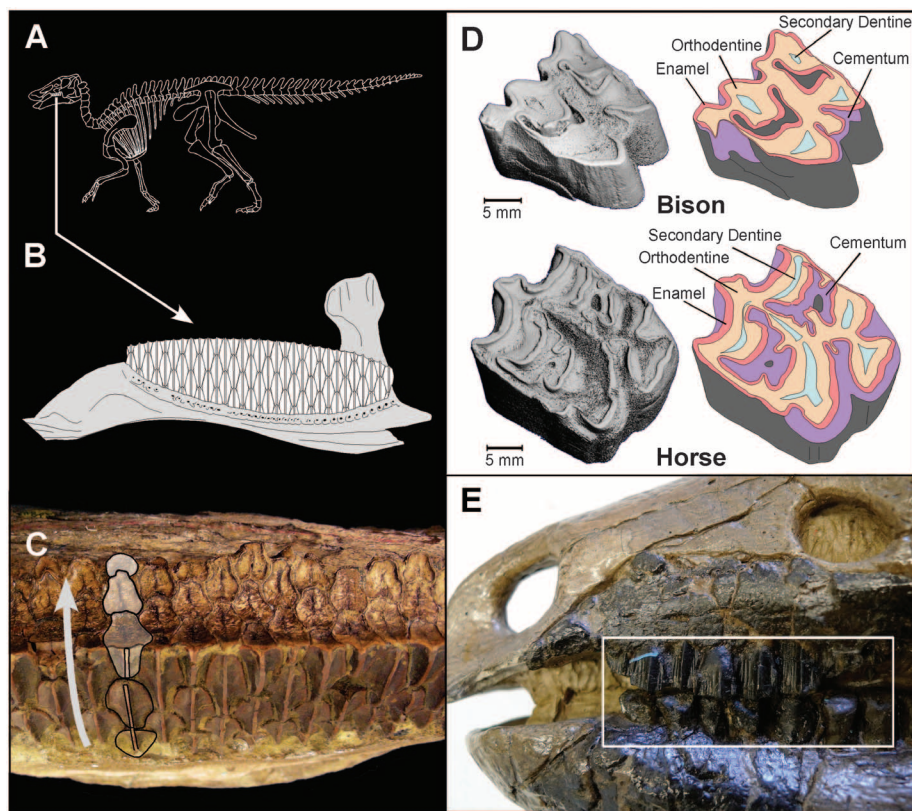


Fig. 1. Dental comparisons. (A) Hadrosaurid skeleton (*Edmontosaurus*). (B) Dental battery showing developing teeth (lingual view). (C) *Edmontosaurus* dental battery showing the progression of developing teeth and the grinding surface with teeth in various wear stages. (D) Ungulate grinding molars showing four-tissue composition. (E) Hadrosaurid outgroup condition (*Camptosaurus*) possessing individual shearing teeth at each position.

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microscopy and micro-computerized tomography (fig. S2).

Relative values for tissue wear rates (a direct measure of material removal) and hardnesses (the resistance of a solid to permanent deformation when loaded) are the most pertinent properties to determine how dental tissues contribute to whole-tooth abrasive wear (18, 19). Before this study, these had not been recovered from fossil teeth. In dried modern teeth, analogs to well-preserved fossils, material properties are commonly recov-

ered (20), because apatite mineral content is the major determinant of osseous tissue hardness (21). In a feasibility analysis, we used nano-indentation hardness testing on extant and Late Pleistocene bison molar tissues, in which tissues were indented with a diamond tip with equal force to provide comparative data (17). This showed comparable relative values between the tissues of fossil and extant teeth (Fig. 3C).

We characterized the hadrosaurid tissue wear properties by subjecting an *Edmontosaurus*

[American Museum of Natural History (AMNH) specimen no. 5896] dental battery to microtribological wear testing, in which a diamond-tipped probe was drawn across the tooth (at a sliding velocity of 1 mm/s, with 100 mN of normal force) to mimic abrasive feeding strokes (17) (fig. S4). A surface profiler measured the volume of material removed, which was divided by the product of the normal force and the sliding distance to reveal the wear rates (17). Indicators that biological values were recovered included the correspondence of wear depths to relief on naturally worn batteries, and the capacity to replicate the chewing surface topography using a three-dimensional tribological simulation (17, 22) based on Archard's wear law (18, 23) (Eq. 1)

$$V(\text{mm}^3) = K[\text{mm}^3/(\text{Nm})]F_n(\text{N})d(\text{m}) \quad (1)$$

{The volume of material lost, V (in mm^3), is equal to wear rate, K [in $\text{mm}^3/(\text{Nm})$], times normal load, F_n (in newtons, N), times sliding distance, d (in meters, m).}

The model numerically simulates topographical changes to a multi-tissue hadrosaurid dental battery subjected to wear with an abrasive compliant pad analogous to hadrosaurid fodder. Starting from a planar surface encompassing tissue distributions and wear rates, the progressive coupling of wear and contact pressure (a function of surface geometry) results in an equilibrium topography where all tissues recede at equal rates. The model was ultimately used to test how tissue distributions act to create surface features through wear.

To confirm that wear-relevant properties are preserved in the 65- to 69-million-year-old fossils, we nanoindented teeth from AMNH 5896 and two others (17). Indicators for preservation include hardnesses correspondent with wear rates, similar values among individuals, and the capacity to predict the chewing surface morphology through the wear model, using hardness as a wear rate proxy (17). We also collected comparative data for the domestic horse (*Equus caballus*).

Results show that hadrosaurid teeth were composed of six major tissues (Fig. 2D). These include all four wear-relevant constituents that characterize mammalian grinding teeth: enamel and orthodentine, as well as independently derived secondary dentine and coronal cementum [a tissue used to demonstrate mammalian dental sophistication (10, 14)]. Giant tubules (infilled pulp cavity branches) and a thick mantle dentine are also present. These results suggest that hadrosaurid teeth were among the most histologically complex of any animal. Additionally, unlike mammalian teeth (11), tissue distribution varied substantially within each tooth, exposing different configurations as they migrated across the chewing surface (Fig. 2, D and E). This allowed a single tooth to assume different forms and functions during its progression.

Tribology experiments revealed wear rates ranging from $\sim 90 \times 10^{-6} \text{ mm}^3/\text{Nm}$ for orthodentine (the least wear-resistant) to $\sim 6 \times 10^{-6} \text{ mm}^3/\text{Nm}$

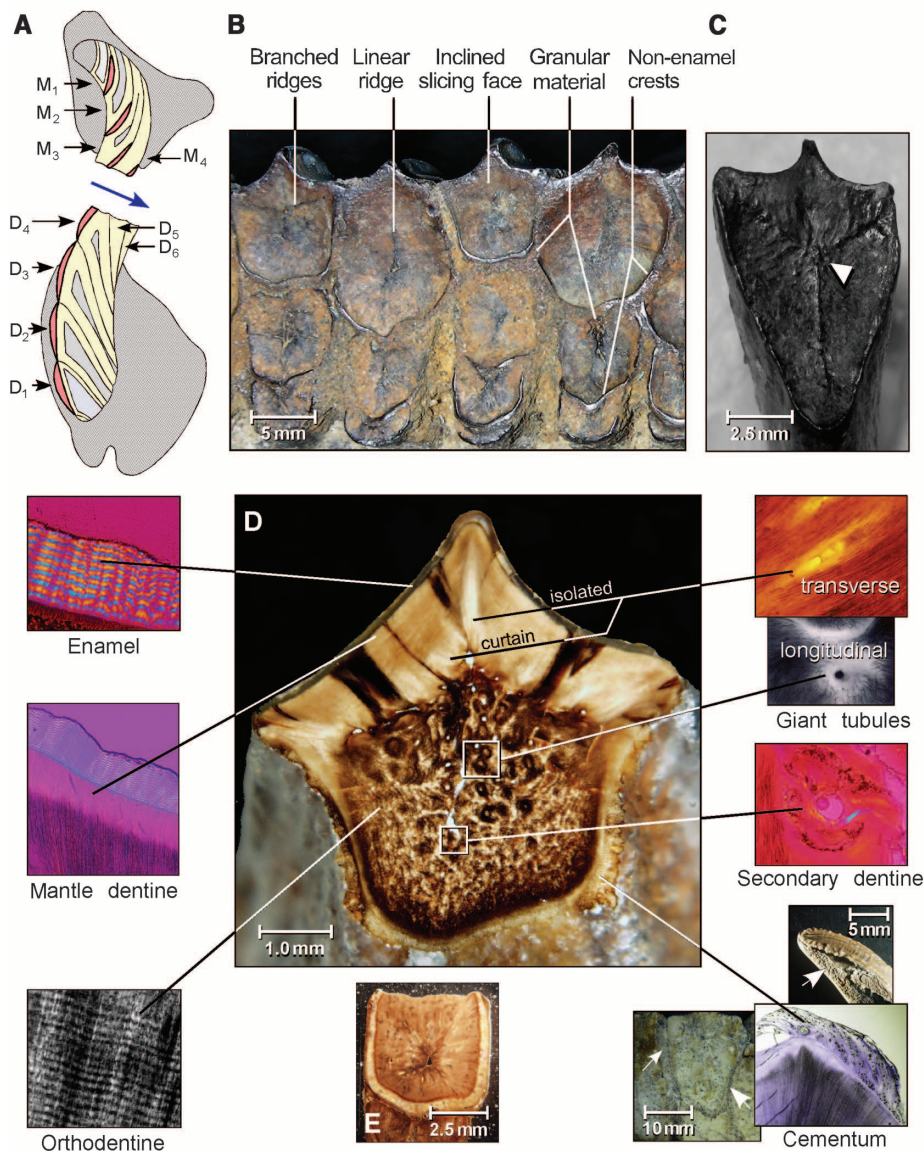


Fig. 2. Hadrosaurid dental organization. (A) Two-tissue model, frontal section of jaws depicting just enamel (red) and dentine (yellow) tooth composition (3, 4), with no enamel layers across the lower chewing surface. The upper (maxillary) teeth and lower (dentary) teeth were drawn at various angles across one another (1). The blue arrow depicts the movement of the upper teeth across the lower ones. The lower teeth may have been simultaneously drawn in the opposite direction. Tooth developmental stages are numbered in the upper (M_1 – M_4) and lower (D_1 – D_6) jaws. [Worn teeth were shed every 45 to 80 days from each column (7), up to 1880/year in total (17).] (B) Unexplained chewing surface features. (C) *Hadrosaurus* tooth with a Y-shaped ridge (arrow). (D) Sections through *Edmontosaurus* teeth showing tissues. Their presence and configurations vary throughout individual teeth. For instance, the roots (E), which become exposed, lack giant tubules. Coronal cementum (arrows) is present on the chewing surface and sides of a crown.

for enamel (the most wear-resistant) (Fig. 3, A, B, and D), which correspond to topographic features on worn batteries (Figs. 1C and 2B). Input of these values into the wear model re-

sulted in a three-dimensional topography seen in naturally worn batteries. This includes crests where enamel is absent, as well as branched ridges (Fig. 4). Simulation reveals the contributions

of each tissue to the worn topography (fig. S5). This shows that the overall morphology is impossible to achieve without the entire tissue ensemble (figs. S5 and S6). These findings

Fig. 3. Wear and hardness characterization. (A) Planarized *Edmontosaurus* tooth with wear track (left). (B) Profilometry of scratched tissues in AMNH 5896. Secondary dentine was not tested owing to its negligible footprint. (C) Mean and standard deviation from nanoindentation experiments on wear-relevant tissues in ungulates and hadrosaurids. The fossil bison absolute hardness values appear to be elevated from the degradation of elastic collagen, but the preservation of relative hardness, which is critical for understanding wear, is evident. Tissue-specific hardnesses in extant ungulate grinding teeth are highly variable among taxa. An exact match between values for the bison or horse and the analogous *Edmontosaurus* tissues is not expected. (D) Mean wear rates and standard deviation versus mean hardness and standard deviation for AMNH 5896 tissues.

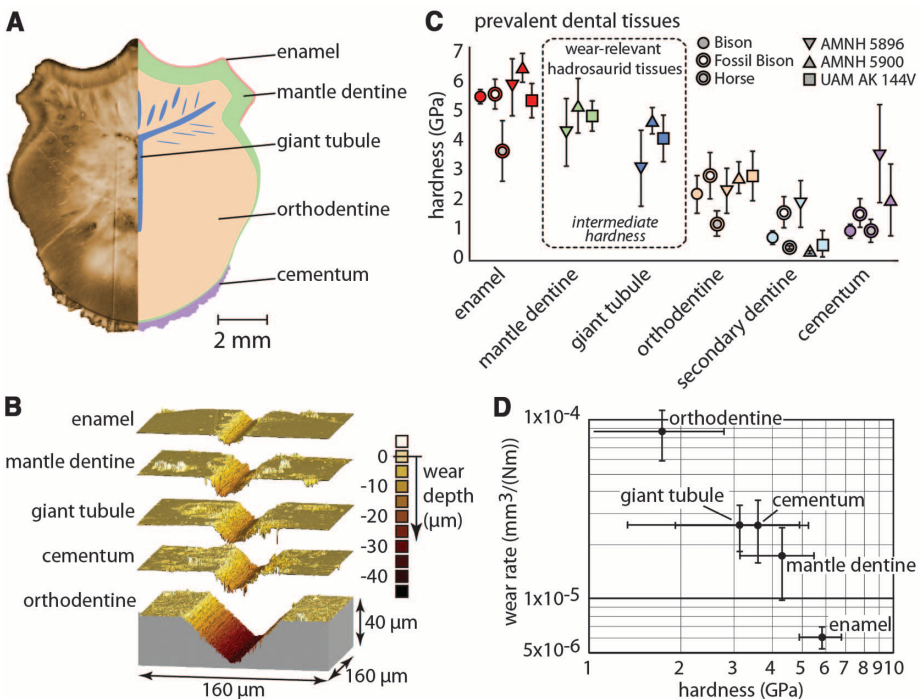
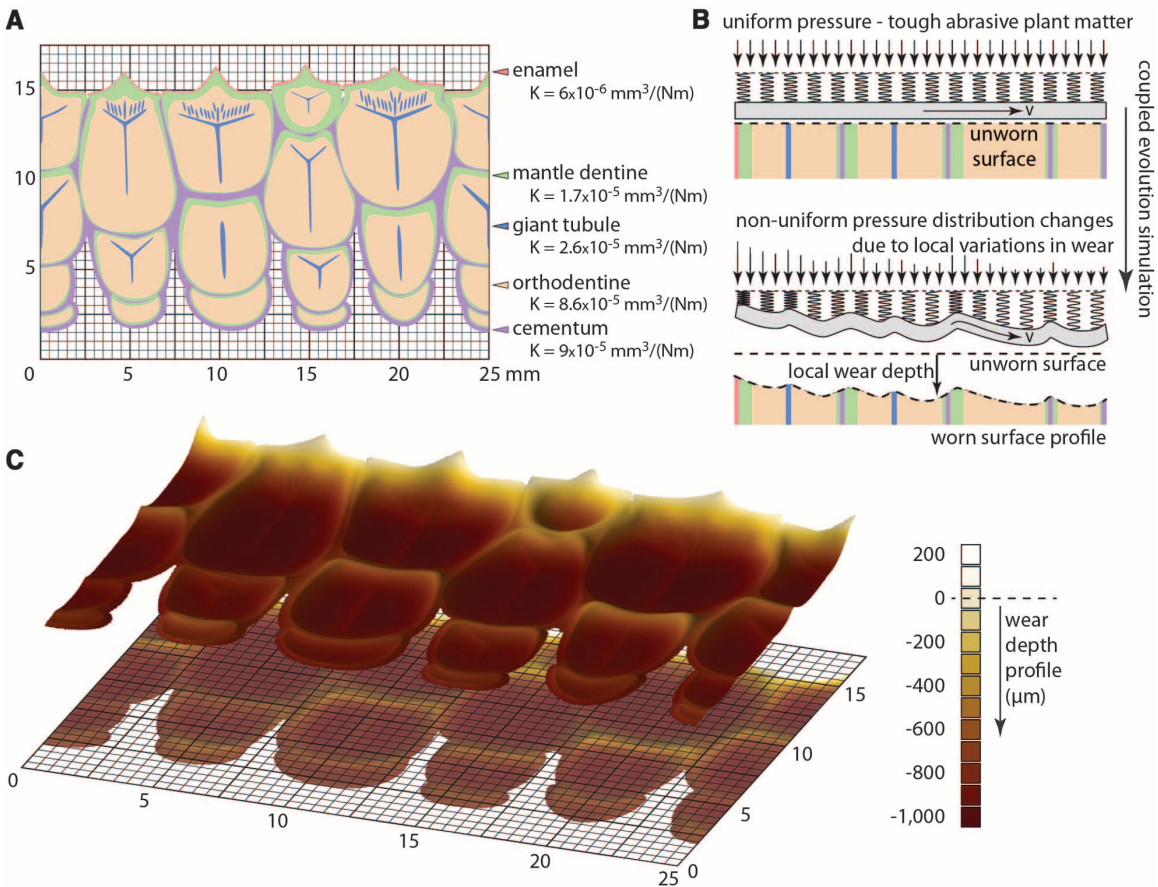


Fig. 4. Tribological modeling of the AMNH 5896 dental battery. (A) Tissue distribution and measured wear rates used in the simulation. (B) Schematic of the computational framework and simulation procedure. An initially flat composite is exposed to abrasive wear under uniform pressure. The progression of wear depth and contact pressure are linked, leading to uniform recession only at a steady state. (C) Equilibrium profile following computational wear modeling of an initially planar composite surface of varying tissue types assigned fossilized wear-rate values.



provide strong evidence that biologically relevant tribological properties are preserved in the fossil teeth.

Nanoindentation revealed mean hardness values from ~1 GPa for secondary dentine to ~6 GPa for enamel (Fig. 3C). Hardness and wear rates are related as expected for AMNH 5896 (Fig. 3D), and hardnesses were similar among *Edmontosaurus* individuals (Fig. 3C). Hardness-based wear models for all three dinosaurs also wore to a morphology matching naturally worn batteries, including crests where enamel is absent and branched ridges (fig. S7).

In a phylogenetic context, these histological, biomechanical, and simulation data demonstrate how hadrosaurids evolved mammal-like grinding capacity. The primitive condition, in Hadrosauridae, seen in *Edmontosaurus* and most taxa (fig. S3), was a dual-function slicing-grinding system, presumably for the consumption of fibrous, moderately tough plants (2). The leading teeth have an inclined slicing plane, whereas all others form a file-like pavement. Highly wear-resistant enamel forms crests in all upper battery teeth but only in the lead teeth in the lower battery, because enamel was worn away before the teeth migrated across the chewing surface (Figs. 2A and 4A). Wear-resistant mantle dentine is the tissue that takes over the crest-forming role in the lower batteries (Fig. 4A and fig. S5). The inclined slicing faces in the leading teeth are composed of giant tubule curtains with intermediate wear resistance, spanning between the wear-resistant mantle dentine crests and the high-wear orthodentine basins (Fig. 2D and fig. S5). Large individual and branched giant tubules formed intermediate-height ridges partitioning the basins (Fig. 4A and fig. S5). Modeling shows that they influenced basin depth at each tooth position [greater sliding distance = greater scour (23)] and probably provided for finer grinding of plants than did major crests (fig. S5). Coronal cementum is prevalent, as in mammalian grinding teeth (Figs. 2D and 4A). It similarly served as a bridge minimizing stress singularities on the hard brittle crests, but also bound teeth together (fig. S5). Abscess-preventing secondary dentine is present only where the pulp cavity was locally breached and, unlike in mammalian grinding teeth, didn't substantially contribute to basin formation through wear.

The distribution of these characters phylogenetically shows that longitudinal giant tubules and secondary dentine evolved at the base of Ornithomimidae, probably for abscess prevention in association with dental occlusion (fig. S3). Transverse giant tubules subsequently appeared in hadrosauroids for steeper-angled slicing. The remaining tissues (mantle dentine and extensive coronal cementum) are primitive for Hadrosaurids and evolved as innovations for combined slicing and grinding (fig. S3). Tissue-complex modifications appear to have allowed for diversification into specialized ecological niches (fig. S3). Some taxa evolved teeth with coarse grinding pavements across the entire chewing area, presumably for processing

tough plant matter (figs. S2 and S3). This was achieved through the loss of transversely oriented giant tubules, so slicing plane formation and basin partitioning couldn't occur. In other taxa, grinding capacity was completely lost and the teeth were specialized for high-angle slicing (fig. S3). In these batteries, transversely oriented giant tubules radiate throughout the teeth, so shearing faces formed at all wear stages across the chewing surfaces.

Hadrosaurids evolved the most histologically and biomechanically sophisticated dentitions known among reptiles, and these rivaled those of advanced herbivorous mammals in complexity. Three-dimensional tribological modeling allows for an improved understanding of tissue-level contributions to dental form and function. The ability to measure wear-relevant properties in fossils provides a new approach to study biomechanics throughout evolution. Such inferences will be enlightening across major mammalian and reptilian diversifications involving dental and dietary changes (24, 25).

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Supplementary Materials

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Rapid Acceleration Leads to Rapid Weakening in Earthquake-Like Laboratory Experiments

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After nucleation, a large earthquake propagates as an expanding rupture front along a fault. This front activates countless fault patches that slip by consuming energy stored in Earth's crust. We simulated the slip of a fault patch by rapidly loading an experimental fault with energy stored in a spinning flywheel. The spontaneous evolution of strength, acceleration, and velocity indicates that our experiments are proxies of fault-patch behavior during earthquakes of moment magnitude (M_w) = 4 to 8. We show that seismically determined earthquake parameters (e.g., displacement, velocity, magnitude, or fracture energy) can be used to estimate the intensity of the energy release during an earthquake. Our experiments further indicate that high acceleration imposed by the earthquake's rupture front quickens dynamic weakening by intense wear of the fault zone.

Large earthquakes initiate at a small nucleation area and grow as propagating rupture fronts (1, 2) (Fig. 1A). The propagating front activates a multitude of fault patches that

undergo intense deformation (Fig. 1, B and C). Before the front arrives, the stress μ on each patch is generally lower than its static strength μ_s [both stress and strength are presented as the friction

coefficient, $\mu = (\text{shear stress}/\text{normal stress})$. If the arriving front raises the stress to the static strength level, the patch strength may drop (1) (Fig. 1B), and it slips while releasing elastic energy stored in the rocks, and eventually it decelerates and stops. This model is widely accepted (1–7), yet the evolution of strength, velocity, and energy partitioning are poorly constrained (2, 4).

In stick-slip experiments (3, 7), the above earthquake sequence was simulated by loading an experimental fault until it spontaneously generated an earthquake-like event (Fig. 1B). However, these experiments are limited to tiny displacements (3, 6, 7) that are five to six orders of magnitude less than large earthquake displacements. To circumvent this limitation, earthquake modelers relied on constitutive equations, like rate- and state-friction, based on steady-state friction data.

We simulated fault-patch slip during large earthquakes by employing one central concept: abruptly

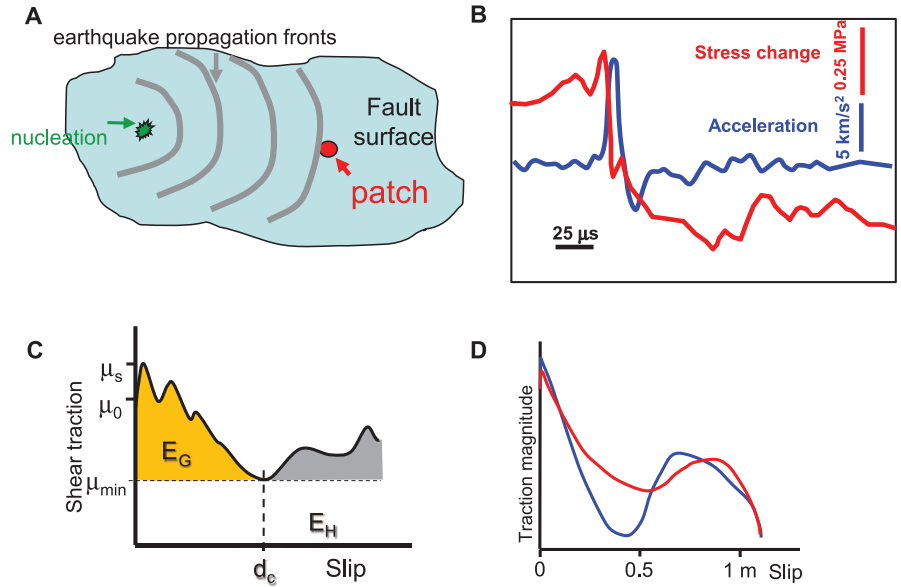


Fig. 1. Stress and energy on a fault patch during an earthquake. (A) A fault patch activated by a propagating earthquake rupture. (B) Shear stress and slip acceleration during a stick-slip event along an experimental granite fault (3). (C) Conceptual evolution of shear traction on a fault patch during an earthquake (2). E_H , frictional heat energy; E_G , breakdown energy. (D) Calculated traction evolution of two fault patches of Northridge earthquake (2).

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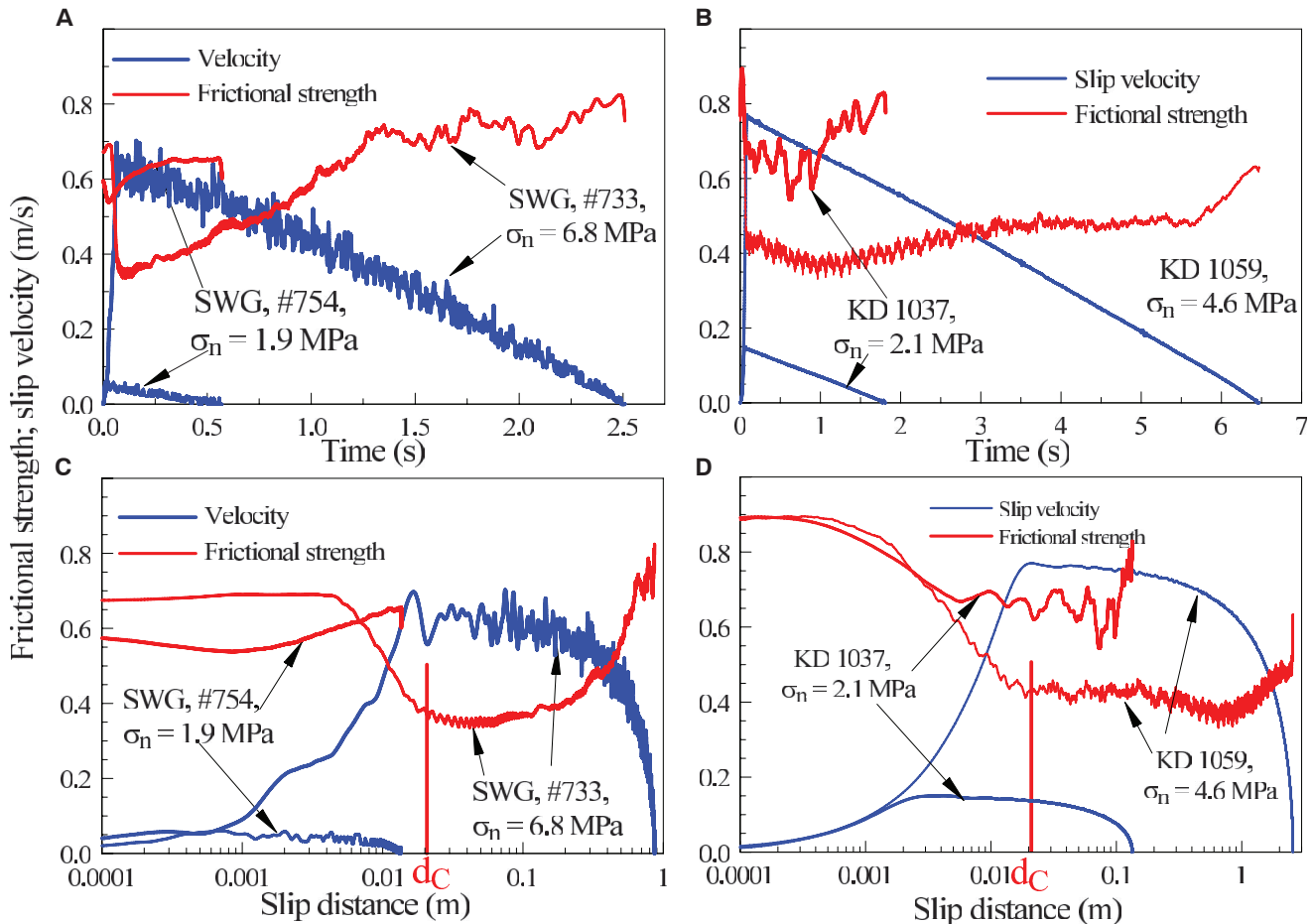


Fig. 2. The evolution of frictional strength and slip velocity in ELSE experiments with respect to (A and B) time and (C and D) slip distance (9). (A and C) Two runs with SWG loaded with $E_c = 0.007$ m (#754) and $E_c = 0.43$ m (#733). (B and D) Two runs with KD loaded with $E_c = 0.09$ m (#1037) and $E_c = 1.20$ m (#1059).

deliver a finite amount of energy to an experimental fault patch that spontaneously dissipates the energy without operator intervention. We used a rotary-shear apparatus, in which a ring-shaped fault slips at velocities of up to 1 m/s, at normal stresses of up to 30 MPa (8, 9). We conducted 42 experiments on Sierra White granite (SWG) samples and 24 experiments on Kasota dolomite (KD) samples. Each experiment starts by spinning a 225-kg disk-shaped flywheel to a prescribed angular velocity; during this stage, the sample remains stationary (9). Then, a fast-acting clutch engages the sample-half, forcing it to rotate with the flywheel until all stored kinetic energy is dissipated. We refer to this experiment as an “earthquake-like slip event” (ELSE) (table S1). The total energy is presented by the Coulomb energy density— E_C = [flywheel kinetic energy/(sample area $\times$ normal stress)]—with units of meters (9) (tables S3 and S4).

We first consider a typical run, in which a SWG sample (#733, Fig. 2A) is sheared under normal stress σ_n = 6.8 MPa, and E_C = 0.42 m. The patch accelerated to peak velocity of V_p = 0.70 m/s in ~ 0.1 s and slipped for 0.86 m during 2.50 s. The initial patch strength (μ_s = 0.66) briefly increased (μ = 0.69) and then dropped to a minimum value of $\mu_{\min}$ = 0.35. Finally, the patch strength recovered to μ = 0.81 as the patch decelerated (Fig. 2A).

Similar patterns of strength evolution were observed for ELSE experiments with the following characteristics (Fig. 2, A to D, and figs. S7 and S8): (i) The initial frictional strength (μ_s) was high and usually showed a brief strengthening stage. (ii) As slip continued, the strength decreased to a minimum value, $\mu_{\min}$, frequently followed by restrengthening during deceleration. (iii) Most ELSE runs (48 out of 66 experiments) displayed substantial dynamic weakening (9) that primarily depends on E_C (tables S3 and S4). Five runs with E_C < 0.001 m did not slip, four runs with E_C < 0.03 m slipped but did not reach weakening, and nine runs displayed negligible weakening. (iv) SWG samples loaded by E_C > 0.1 m displayed high-frequency stick-slip behavior; this mode was less common in KD samples. (v) The total slip distance, D , and the Coulomb energy density are related by $E_C = 0.605 \times D^{0.933}$ for both granite and dolomite experiments, over almost four orders of magnitude (Fig. 3A).

This strength evolution in ELSE runs is similar to strength evolution in earthquake models (Fig. 1C), stick-slip experiments (Fig. 1B), variable-rate experiments (10), and seismic analyses (Fig. 1D). To test this similarity, we assumed that the measured slip in ELSE experiments is equivalent to the average slip, $\bar{D}$, during an earthquake. Then, we used empirical relationships (11) between the moment

magnitude (M_w) and $\bar{D}$, to find that ELSE (D = 0.003 to 4.6 m) corresponds to earthquakes in the range M_w = 4 to 8 (upper axis, Fig. 3A). Further, two other experimental parameters—peak velocity (1 m/s) and rise time (0.1 to 10 s) (Fig. 3A)—have values similar to the equivalent parameters of earthquakes (2). It appears that ELSE runs are similar to earthquakes in three ways: (i) a finite amount of stored energy; (ii) fault strength evolution pattern (Figs. 1D and 2); and (iii) seismically observed values, such as average slip, peak velocity and rise time (Fig. 3A). We propose that ELSE tests are experimental proxies for fault-patch behavior during earthquakes of M_w = 4 to 8 and now apply ELSE results to the questions of total earthquake energy and weakening distance (9).

To examine whether the experimental E_C is a reasonable estimate of the total earthquake energy, we compared experimental E_C to the breakdown (or fracture) energy (E_G) of six earthquakes (2) of M_w = 5.6 to 7.2 (9) (table S2). Breakdown energy is only a fraction of the total earthquake energy (2, 6, 9). As we did with our representation of E_C , we divide the seismic E_G by the corresponding σ_n (9), and the E_G/σ_n values are plotted with respect to their slip distance (vertical lines, Fig. 3A). Most (9 out of 11) of seismic E_G/σ_n values are small fractions (0.005 to 0.07) of the energy density of ELSE, as expected for

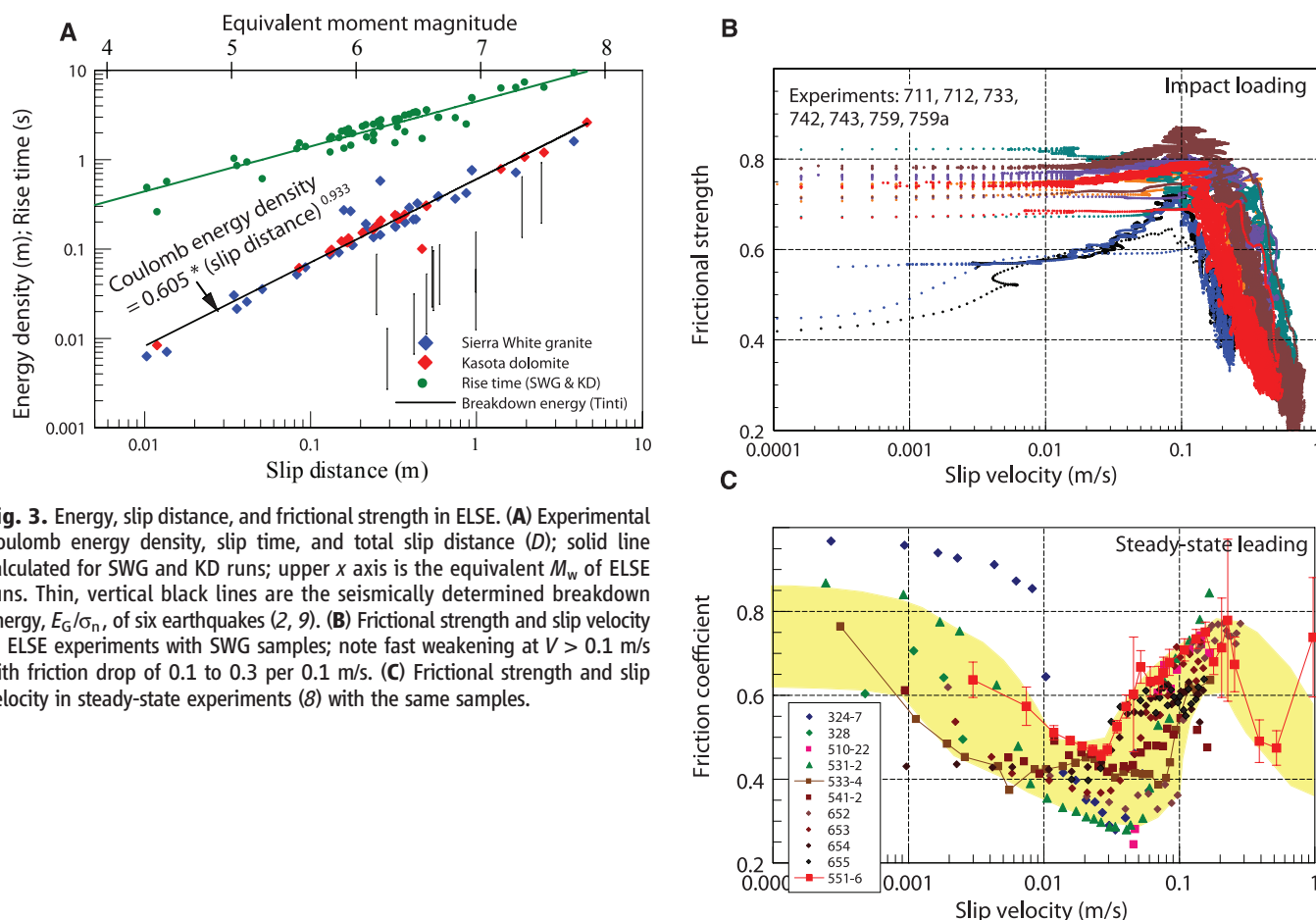


Fig. 3. Energy, slip distance, and frictional strength in ELSE. (A) Experimental Coulomb energy density, slip time, and total slip distance (D); solid line calculated for SWG and KD runs; upper x axis is the equivalent M_w of ELSE runs. Thin, vertical black lines are the seismically determined breakdown energy, E_G/σ_n , of six earthquakes (2, 9). (B) Frictional strength and slip velocity in ELSE experiments with SWG samples; note fast weakening at $V > 0.1$ m/s with friction drop of 0.1 to 0.3 per 0.1 m/s. (C) Frictional strength and slip velocity in steady-state experiments (8) with the same samples.

fracture energy (6, 9, 12, 13). The energy budget measured locally on a fault patch does not include radiated energy (4), and radiated energy is generally considered small compared with dissipated energy. We thus speculate that the experimental Coulomb energy density, $E_C = 0.605 \times D^{0.933}$, provides a reasonable estimate of total earthquake energy, a quantity that cannot be determined from seismic data (2, 4).

We now consider slip-weakening distance, d_C (Fig. 1, C and D). In friction experiments, d_C ranges from microns in direct shear (3, 6, 7) to meters in rotary shear (8, 10, 14, 15). We identified d_C as the first slope break of the strength-displacement curve (Fig. 2, C and D). The 49 ELSE runs that display substantial dynamic weakening reveal d_C with mean values of 2.7 and 1.2 cm for SWG and KD, respectively. We attribute these d_C values, which are smaller than in most other rotary tests, to ELSE loading style. The fault patch in ELSE is abruptly loaded by engaging the spinning flywheel through a fast-acting clutch, and this impact loading profoundly affects the patch response. In ELSE runs with SWG samples (Fig. 3B), the frictional strength is 0.6 to 0.8 for $V < 0.1$ m/s, and it drops sharply to as low as

$\mu \approx 0.2$ for $V > 0.1$ m/s. In contrast, our constant-velocity, steady-state experiments (8) (Fig. 3C) exhibit strikingly different friction-velocity relations. Because both sets of experiments were conducted on the same rock samples, the dissimilarity is attributed solely to the different loading mode, as already observed by (10). We further noted that fault strength and wear rate (9) evolve systematically with slip acceleration: A close correlation exists between the evolutions of acceleration (blue), strength (red), and wear rate (black) (Fig. 4 and figs. S7 and S8). In addition, dynamic weakening is restricted to the period of intense acceleration (up to 25 m/s^2 during ~ 0.1 s) (Fig. 4, A and B, and figs. S9 and S10), and d_C is reached within the initial acceleration spike.

The above relations of acceleration and weakening distance were previously observed in stick-slip (3) (Fig. 1B) and rotary-shear experiments (9, 10, 16, 17) (fig. S5). The constitutive relations between acceleration, velocity, and critical distance were derived with the cohesive-zone model (3, 18), which assumes that the extreme acceleration and stress at the tip of a propagating shear rupture leads to fault breakdown. ELSE results fit well the constitutive relations predicted by the cohesive-zone

model (9) (Fig. 4C). Stick-slip and ELSE experiments (elastic versus kinetic energy) fit the same theoretical model, which suggests that the acceleration effect does not depend on details of the loading system.

What is the mechanism of cohesive-zone breakdown? Experimental stick-slip events (3) display intense acceleration ($< 4 \text{ km/s}^2$), which induces extreme strain rates ($\sim 10^4 \text{ s}^{-1}$) that increase rock brittleness and fragmentation (19, 20). These processes temporarily intensify fault wear as manifested by high initial wear rates of 10^2 to $10^4 \text{ } \mu\text{m/m}$ (Fig. 4 and figs. S9 and S10). This intense wear generates a layer of fine-grain powder (gouge) (8, 10, 16) that reduces the fault strength by powder lubrication (8, 14, 20, 21). Thus, slip-acceleration speeds up fault wear and gouge formation and by doing so quickens weakening and shortens d_C . Yet, the accelerated weakening does not change the steady-state friction (15). Accelerated weakening is likely to be active in earthquakes where fault patches are accelerated by the rupture front (Fig. 4C).

It is generally accepted that earthquake dynamic weakening is determined by slip distance and slip velocity (2–5, 8, 14–16). We experimen-

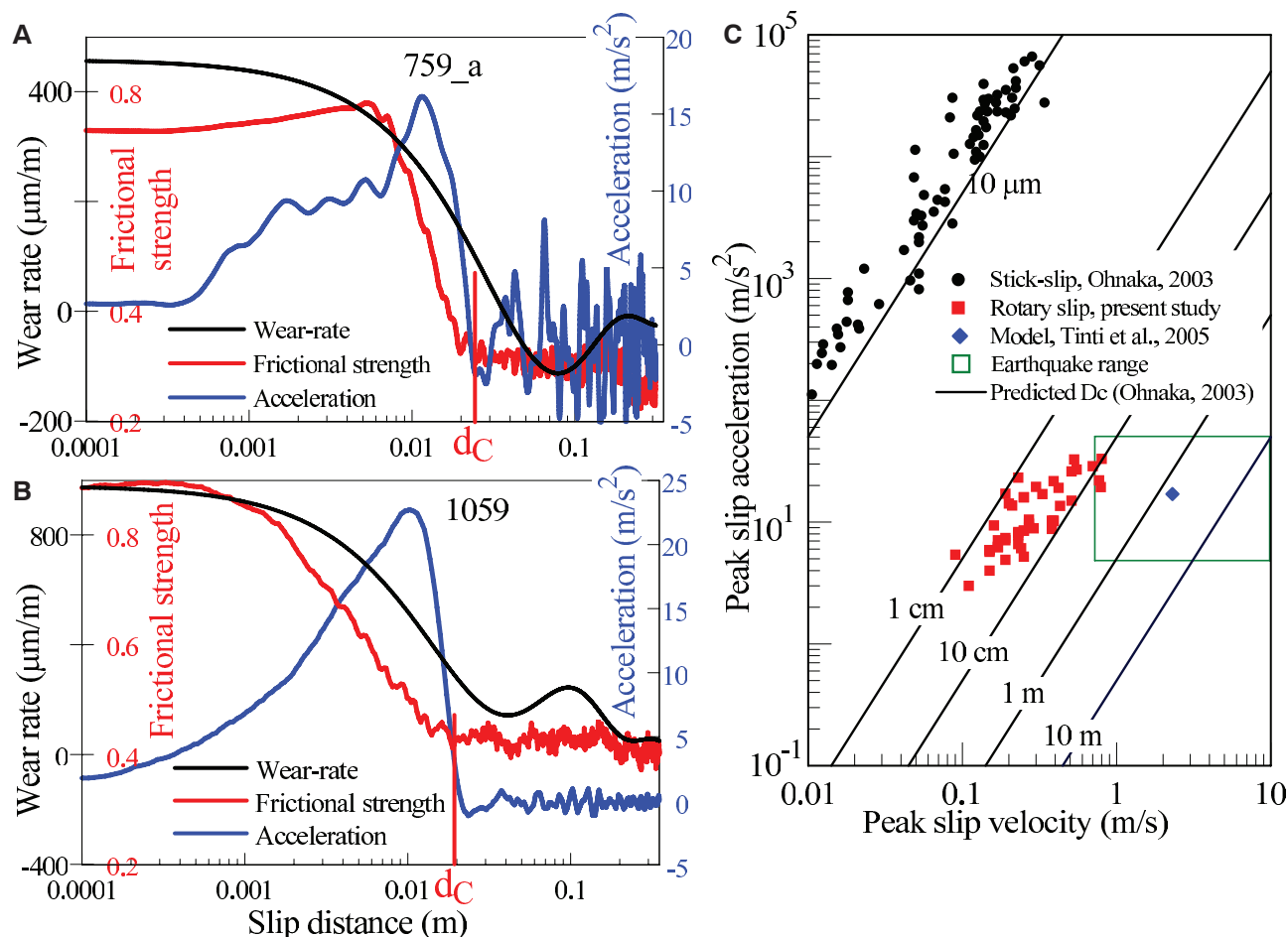


Fig. 4. Slip acceleration (blue), strength evolution (red), and fault wear rate (black) during the first 0.5 s of slip in SWG sample (A) and KD sample (B) (9). (C) Acceleration-velocity- d_C relations according to the cohesive-zone

model (3, 9). The inclined lines indicate the expected d_C (9); the projected ELSE data (red points) fall in the $d_C = 1$ to 10 cm range that fits the experimental range of $d_C = 0.3$ to 9.2 cm.

tally demonstrated that slip acceleration quickens fault weakening, and, in light of the transient nature of earthquake slip (1–3), we propose that slip acceleration controls seismic weakening in addition to slip distance and slip velocity.

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Supplementary Materials

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Materials and Methods
Supplementary Text
Figs. S1 to S10
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The Efficacy of Student-Centered Instruction in Supporting Science Learning

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Transforming science learning through student-centered instruction that engages students in a variety of scientific practices is central to national science-teaching reform efforts. Our study employed a large-scale, randomized-cluster experimental design to compare the effects of student-centered and teacher-centered approaches on elementary school students' understanding of space-science concepts. Data included measures of student characteristics and learning and teacher characteristics and fidelity to the instructional approach. Results reveal that learning outcomes were higher for students enrolled in classrooms engaging in scientific practices through a student-centered approach; two moderators were identified. A statistical search for potential causal mechanisms for the observed outcomes uncovered two potential mediators: students' understanding of models and evidence and the self-efficacy of teachers.

The need for a different approach to science teaching and learning has been the focus of several recent policy and economic reports (1, 2). Research as synthesized by the National Research Council suggests that the goal of science instruction should be to help students develop four aspects of scientific proficiency, the ability to (i) know, use, and interpret scientific explanations of the natural world; (ii) generate and evaluate scientific evidence and explanations; (iii) understand the nature and development of scientific knowledge; and (iv) participate productively in scientific practices and discourse (3–5). This approach to science teaching will require a shift from the teacher-centered instruction common in science classrooms to more student-centered methods of instruction. The defining feature of

these instructional methods is who is doing the sense-making. In teacher-centered instruction, the sense-making is accomplished by the teacher and transmitted to students through lecture, textbooks, and confirmatory activities in which each step is specified by the teacher. In these classrooms, the instructional goal is to help students know scientific explanations, which is only part of the first aspect of scientific proficiency. In student-centered instruction, the sense-making rests with students, and the teacher acts as a facilitator to support the learning as students engage in scientific practices (3).

The effectiveness of student-centered instruction in helping students develop scientific proficiency is supported by a number of largely small-scale, narrowly focused studies (3, 5). Despite accumulating support for a student-centered approach, few large-scale studies have evaluated the effectiveness of such instruction, and their results, taken as a whole, are contradictory and inconclusive (6–13). The same is true of only randomized-cluster or quasi-randomized studies examined separately (6, 11, 14, 15). Many factors may contribute to the varied results, because

tightly controlling potentially influential variables is difficult in classroom settings. One central factor is that the comparison condition (i.e., control group) is often “undefined or assumed to be ‘traditional’” (14). Likewise, possible “contamination of the untreated teachers” and cases where investigators did not “vigorously guard” against special resource materials may have influenced results (13). Indeed, many studies described in the literature do not discuss how fidelity to the curriculum or instructional approach was measured or whether it was assessed.

We therefore compared the effectiveness of student-centered with teacher-centered instruction using a randomized-cluster experimental design, intended to control as many variables as possible given the inherent differences between the two instructional approaches. Specifically, the effectiveness of the student-centered Great Explorations in Math and Science Space Science Curriculum Sequence (SSCS) (16) and professional development of teachers focused on these materials (treatment group) was compared with that of a teacher-centered curriculum (district-adopted textbook) enacted with a teacher-centered approach (control group). For details of each curriculum, teacher professional development, and instructional approach, see the supplementary materials. Mindful of limits on securing meaningful data imposed by testing the age group for whom SSCS is appropriate (fourth and fifth grades), we selected four student outcomes aligned with the four aspects of scientific proficiency for this research: space science content knowledge, knowledge about models and evidence in science, views of scientific inquiry, and attitude toward science. The research was designed to (i) compare the effectiveness of the two instructional approaches in supporting elementary students' science learning; (ii) identify teacher characteristics (teacher moderating variables) that might influence the learning; (iii) identify those for whom this instructional approach might work (student moderating variables); and (iv) identify how the treatment might indirectly affect student outcomes (mediating variables).

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Data were collected from 125 fourth- and fifth-grade classrooms. Randomization occurred at the level of assignment of teachers to treatment or control group; control and treatment groups were matched according to grade level, socioeconomic status (SES), school statewide assessment performance, and student ethnic diversity. Student demographics were collected (table S1). Contexts included urban, suburban, and rural and high- and low-SES schools; 2594 students participated—1418 in the classrooms of 66 treatment teachers and 1176 in the classrooms of 59 control teachers (for details, see the supplementary materials). Student conceptual development and affective dimensions were assessed by means of four instruments: (i) Space Science Content Test (17); (ii) Homerton Science Attitudes Survey (18); (iii) Models and Evidence Questionnaire (19); (iv) Views of Scientific Inquiry (VOSI) Elementary Version Questionnaire (20). Both groups were assessed immediately before the unit, immediately after the unit, and 5 months $\pm$ 2 weeks after the unit (see the supplementary materials for test and scoring details). Each teacher's fidelity to the assigned teaching approach was assessed with the Reformed Teacher Observation Protocol (RTOP) (21) two to three times during the unit. RTOP is a measure of the degree to which lesson enactment is aligned with student-centered science instruction. To help identify potential teacher moderators, we also assessed teachers' space-science content knowledge, science attitudes, views of scientific inquiry, self-efficacy, and beliefs about science teaching before and after their participation in the project.

Multilevel (hierarchical linear) modeling was used to estimate the SSCS's effects with moderation and to account for interdependencies of student outcomes within teachers (22, 23). Each student outcome was reflected in two measures, the postmeasure and delayed postmeasure. Potential explanatory variables for each outcome included (i) pretest measure; (ii) treatment variable (SSCS or control); (iii) teacher cohort (year 1 or 2); (iv) grade level (4 or 5); (v) interactions among the treatment, cohort, and grade variables; (vi) teacher years of experience; (vii) preassessments of teacher outcomes; (viii) student ethnicity; (ix) student SES, based on participation in the free or reduced lunch program (FRL); (x) student gender; and (xi) primary language of the student. Inspection of bivariate correlations and backward elimination processes produced the final models. Special attention was given to the possibility of interactions involving student and teacher moderators. Multilevel mediation models addressed the question of how the treatment might indirectly affect student outcomes (mediating variables) by two analytic approaches: an application of multilevel modeling based on separate models for each of the variables explained by the model and a simultaneous solution by multilevel path analysis (24). Positive mediation results should be considered as evidence that the data are consistent with the hypothesized mediation. To com-

pare effect sizes for outcomes with different scales, we also present effects in standardized form [0.2 is small, 0.5 medium, and 0.8 large (25)] but note that a small effect should not be interpreted as trivial (15, 25). The VOSI outcome could not be similarly standardized, because it is an ordered binary variable with two levels. For VOSI, a nonlinear version of multilevel modeling describes effects as an odds ratio (i.e., the odds of SSCS students giving a response coded as "transitional/informed" rather than "naïve" divided by the odds of control students doing so); a ratio of 1.1 is at the threshold of practical importance. (See the supplementary materials for details of statistical analysis.)

Average RTOP scores for SSCS teachers were 27.3 points (out of 100) higher than those of con-

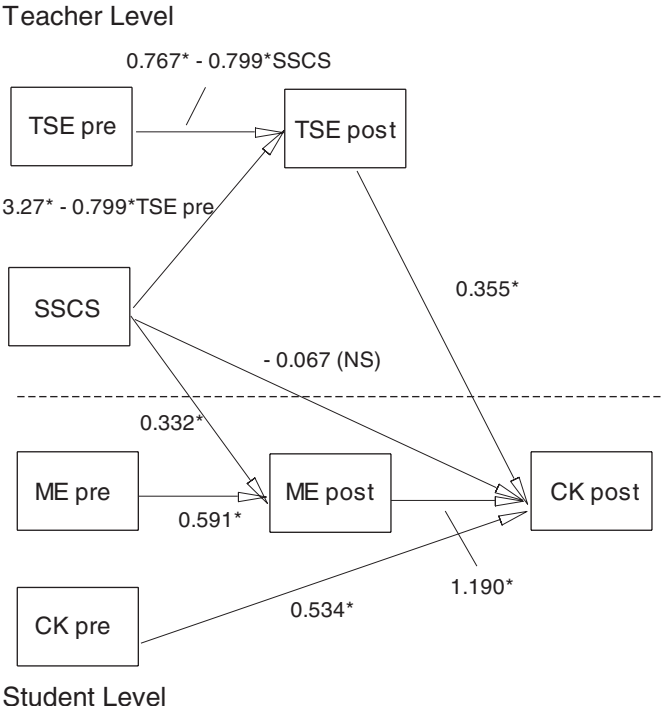
trol teachers, a statistically significant difference ($P = 0.001$); groups overlapped only modestly, indicating that the two instructional approaches were implemented with fidelity. To examine within-group RTOP effects, we transformed total RTOP scores to within-group deviation scores. For each student outcome for which the SSCS effect was statistically significant (content knowledge, models and evidence, and VOSI) (see Table 1), the effect of the RTOP deviation score was positive and statistically significant ($P = 0.002$ to 0.017). That is, after the SSCS treatment effect was controlled for, the RTOP deviation score could be considered as a "dosage" variable within each group resulting in an increase in student outcomes. This finding suggests that student engagement in

Table 1. SSCS total effects for student outcomes. OR, odds ratio.

Outcome	Unstandardized effect	P	Standardized effect†
<i>Posttest outcome</i>			
Content knowledge‡	0.488*	0.002	0.171*
VOSI§	0.464*	0.002	OR = 1.59
Models and evidence			
No FRL	0.354*	<0.001	0.682*
FRL	0.261*	<0.001	0.503*
Attitude toward science	0.009	0.753	0.014
<i>Delayed posttest outcome</i>			
Content knowledge‡	0.187	0.193	0.067
VOSI	0.333*	0.015	OR = 1.40
Models and evidence	0.285*	<0.001	0.573*
Attitude toward science	0.020	0.530	0.029

*Statistically significant, with a family-wise error rate of 0.10 (i.e., for a test family of four post-outcomes or four delayed post-outcomes, $P < 0.10/4 = 0.025$). †For all outcomes except VOSI, standardized effects were obtained by division of raw-score SSCS coefficients by the outcome standard deviations. ‡Results are from a sample from which one extreme outlier was removed. ||The student variable of VOSI is dichotomous. The associated unstandardized SSCS effect is the SSCS coefficient in a model for the log odds (logit) of the outcome, and the standardized SSCS effect is the OR for the transitional/informed outcome.

Fig. 1. Mediation model for student posttest content knowledge, with path coefficients indicated. *, significant at the 0.05 level; NS, not significant; TSE pre, teacher pretest self-efficacy; TSE post, teacher posttest self-efficacy; ME pre, student pretest models and evidence; ME post, student posttest models and evidence; CK pre, pretest student content knowledge; CK post, posttest student content knowledge.



learning (student-centeredness), as indicated by RTOP scores, is a feature of more effective teaching.

Table 1 summarizes estimated total effects of the SSCS curriculum on student post- and delayed posttest outcomes, including student-level moderators. When a family-wise error rate of 0.10 was controlled for, students in the SSCS group scored significantly higher than control students on content-knowledge, models-and-evidence, and VOSI posttest outcomes. For delayed posttests, the SSCS group remained significantly higher for VOSI and models and evidence. For content-knowledge and models-and-evidence outcomes, the standardized effect magnitudes were ~ 0.2 and 0.7, respectively. The odds ratio representing the SSCS effect for the VOSI outcome was 1.59; that is, the odds of SSCS students giving transitional/informed responses were 1.59 times greater than those for control students. Interpreting the results of the content-knowledge delayed posttest requires

accounting for factors potentially affecting it; foremost is the timing, which placed these assessments within about 2 weeks of statewide assessments and all their concomitant drill and practice in both treatment and control classrooms. The large size (25) and persistence of the models-and-evidence and VOSI outcomes are notable.

Only one student characteristic, SES, moderated the SSCS effect on one posttest outcome, models and evidence (Table 1). Although the two SES groups differed in achievement, both high- and low-SES students in the treatment group performed better than did students in the control group. This difference between the groups in the SES achievement gap disappeared by delayed posttesting. This result is consistent with a wide body of research that indicates that students from low-SES groups initially need more support to participate in the practices of science (here, using models and evidence) (6, 11, 26).

Only one teacher characteristic, pretest self-efficacy, moderated one student posttest outcome, content knowledge. Self-efficacy, a well-researched construct (see the supplementary materials for more details), is defined as a teacher's "judgement of his or her capabilities to bring about desired outcomes of student engagement and learning" (27). The SSCS effect was positive and large for low values of teacher pretest self-efficacy but decreased as teacher pretest self-efficacy increased (table S2). That is, students in classrooms of teachers who had low teaching self-efficacy at the outset of the study showed a statistically significant increase in their posttest content-knowledge scores, whereas students in classrooms with teachers who had high initial self-efficacy did not. Much research has examined how teachers' knowledge, attitudes, and beliefs about their abilities shape and are shaped by their classroom experiences [e.g., (28)]; our results suggest that teachers' underlying beliefs, such as self-efficacy, might influence the overall effectiveness of a student-centered curriculum. No teacher moderators were apparent in the delayed posttest results.

A search for possible indirect mechanisms by which the treatment produced its effects on posttest student outcomes resulted in two mediation models. In the first (Fig. 1), both teacher self-efficacy and student models-and-evidence variables mediate the SSCS effect on the student posttest content-knowledge outcome. Indices indicated a good fit of this model to the data. The indirect effect mediated by the teacher posttest self-efficacy measure varied with the level of its pretest measure, being positive and large for low values of teacher pretest self-efficacy but decreasing with increasing levels of the teacher pretest self-efficacy. The indirect effect mediated by student posttest models and evidence was positive, constant, and relatively strong. In this model, the estimated direct effect of the SSCS treatment on student achievement was not statistically significant, so the total effect consisted entirely of the indirect effects of teacher self-efficacy and student understanding of models and evidence. The results of this analysis therefore suggest that the two mediators influence the student content-knowledge outcome somewhat equally in classrooms taught by teachers with lower self-efficacy at the beginning of the project, but the posttest models-and-evidence outcome was the only significant mediator in classrooms taught by teachers who began the project with relatively high levels of self-efficacy (Fig. 2). (See the supplementary materials for more details.)

These results suggest that an emphasis on models and evidence supports students' learning about space-science content. The models-and-evidence instrument was not designed to assess knowledge about models separately from knowledge about evidence, so their individual influences cannot be separated. SSCS students explicitly learned about the nature of both models and evidence in science, as did control-group students, but SSCS students further engaged in activities in

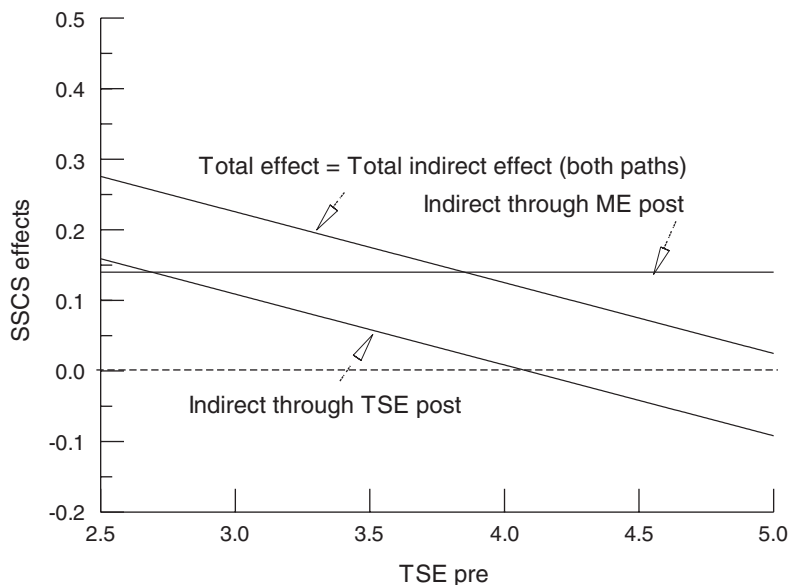
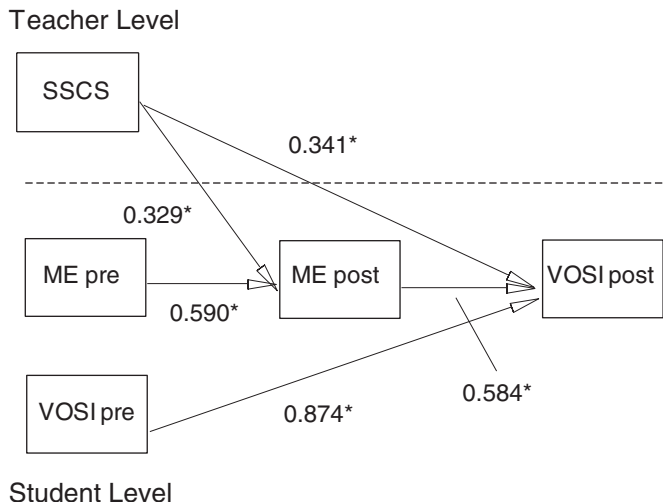


Fig. 2. Standardized effects of SSCS on the student posttest content knowledge outcome. Abbreviations as in Fig. 1.

Fig. 3. Mediation model for posttest VOSI, with path coefficients indicated. Model is for the log odds (logit) of student posttest VOSI. *, significant at the 0.05 level; VOSI pre, pretest student VOSI; VOSI post, posttest student VOSI; other abbreviations as in Fig. 1.



which they used and evaluated models. They also were required to provide explicit evidence that supported new science concepts throughout the curriculum, including activities in which they used evidence to support their arguments about scientific explanations. Experience with models and evidence is therefore supported as underpinning content-knowledge learning.

In the second mediation model, the models-and-evidence outcome mediates the SSCS effect on views of scientific inquiry (Fig. 3). This indirect effect was somewhat smaller than the direct effect of SSCS, but the odds ratio of 1.21 for the indirect path was nevertheless large enough to be of practical importance. The results of this analysis suggest that the emphasis on models and evidence supports students' learning about the endeavor of science.

These findings should also be considered in light of the contradictory results of the previous large-scale studies on the effects of student-centered instruction. In contrast to the common research practice of comparing the treatment group to a control group in which the instructional approach is not specified (i.e., to whatever else is present in schools), our research design tightly controlled the curriculum and instructional approach employed by the treatment and control groups. Further, fidelity was monitored through classroom observations and assessed with RTOP. We argue that this attention to the control group and our efforts to monitor fidelity within both groups sets our study apart from others, and the failure to identify central components of the control group may account for the contradictory nature of previous results.

The increased outcomes of the treatment group in comparison with the control group in content knowledge, models and evidence, and VOSI and the size and persistence of the latter two indicate that student-centered instruction supports the development of students who are more proficient in the four strands of scientific proficiency. More specifically, the mediation models suggest that student-centered instruction that engages students in scientific practices such as using models and evidence is important for developing more scientifically proficient students. Taken together, the results of our study lend empirical support to the view put forth by the National Research Council that "teaching content alone is not likely to lead to proficiency in science, nor is engaging in inquiry experiences devoid of meaningful science content. In current practice, content and an oversimplified view of scientific processes are often the primary or even sole foci of instruction...[and] leads to a very impoverished understanding of science and masks the complex process involved in developing scientific evidence and explanations" [(3), p. 335].

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Supplementary Materials

www.sciencemag.org/cgi/content/full/338/6103/105/DC1
Materials and Methods
Fig. S1
Tables S1 and S2
References (29–37)
Student Data Archive

23 April 2012; accepted 16 August 2012
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Wnt5a Potentiates TGF- β Signaling to Promote Colonic Crypt Regeneration After Tissue Injury

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Reestablishing homeostasis after tissue damage depends on the proper organization of stem cells and their progeny, though the repair mechanisms are unclear. The mammalian intestinal epithelium is well suited to approach this problem, as it is composed of well-delineated units called crypts of Lieberkühn. We found that Wnt5a, a noncanonical Wnt ligand, was required for crypt regeneration after injury in mice. Unlike controls, Wnt5a-deficient mice maintained an expanded population of proliferative epithelial cells in the wound. We used an *in vitro* system to enrich for intestinal epithelial stem cells to discover that Wnt5a inhibited proliferation of these cells. Surprisingly, the effects of Wnt5a were mediated by activation of transforming growth factor- β (TGF- β) signaling. These findings suggest a Wnt5a-dependent mechanism for forming new crypt units to reestablish homeostasis.

Tissue regeneration requires proper spatial allocation and organization of stem cells for efficient return to homeostasis (1, 2). Crypts of Lieberkühn are subunits that house intestinal stem cells and are lost in response to a variety of insults, including ischemia, infection, irradiation, and inflammatory bowel disease (3). Although individual crypts undergo

fission to replicate during homeostasis (fig. S1A) (4, 5), the mechanism of their regeneration is unknown. Thus, crypt regeneration is a proxy for proper stem cell organization and provides an excellent system to uncover the principles underlying stem cell replacement and/or organization *in vivo*.

To model crypt/epithelial stem cell loss, we previously developed an injury system to focally

excise crypts from the absorptive inner lining of the mouse colon (6). In response to the excision of $\sim 1\text{-mm}^2$ areas from the inner lining of mouse colons (~ 250 to 300 crypts), a reproducible program of epithelial alteration occurred. During the first phase (0 to 4 days postinjury), a flattened layer of nonproliferative epithelial cells [wound-associated epithelial (WAE) cells] emanated from crypts adjacent to the wound and migrated over the wound-bed surface. During the second phase (4 to 8 days postinjury), crypts adjacent to the wound formed lateral, open extensions toward the center of the wound bed, forming an array of

channel-like structures (fig. S1, B and C). Histologic cross sections of wound channels at day 6 postinjury showed that they resembled crypts (Fig. 1A) but were distinguished by a predominately proliferative, undifferentiated cell population (Fig. 1B and fig. S1, D and E) (7).

We hypothesized that new crypts within wounds arose from existing crypts adjacent to the wound bed (6). Therefore, we used *Vil-CreERT: Gtosa26^{tm1Sor} (Rosa26R)* mice to perform lineage-tracing experiments (see the supplementary materials and methods) (8, 9). To mark a subset of crypts before injury, we activated Cre recombinase in the intestinal epithelium with a single tamoxifen injection, followed by a 1-week delay. Four days postinjury in these mice, coherent columns of LacZ-positive WAE cells emanated from adjacent crypts toward the wound center (Fig. 1C and fig. S2A) (6). At day 8 postinjury, distinctive LacZ-positive epithelial channels emanated from adjacent crypts toward the center of the wound (Fig. 1, C and D, and fig. S2A). At later time points (14 and 28 days postinjury), clusters of LacZ-positive crypts were located within the original wound-bed site (Fig. 1,

C and E). Sections of these LacZ-positive crypts showed a return to homeostasis; differentiated epithelial cells (i.e., goblet cells) were present in the upper crypt, similar to crypts in nonwounded areas (fig. S2B). These experiments showed that new colonic crypts can originate from crypts adjacent to the wound site and suggest that epithelial stem cells migrated through distinctive channels to enter the wound bed (fig. S2C).

At day 6 postinjury, we readily observed multiple invaginations of wound channels, suggesting that multiple fission events occurred to produce new crypts (Fig. 1A). Comparison of transcript abundance (10) of budding wound channels (day 6 postinjury) to crypts in uninjured areas showed that the wound-channel epithelium contained highly proliferative cells (fig. S3A) enriched in Wnt signaling transcripts (canonical and noncanonical combined) (fig. S3, A and B). As canonical Wnt signaling is critical for intestinal stem cell homeostasis (11–14) and noncanonical Wnt signaling plays a role in intestinal development (15), we screened expression of 19 Wnt ligands and 4 R-spondin coactivators by reverse

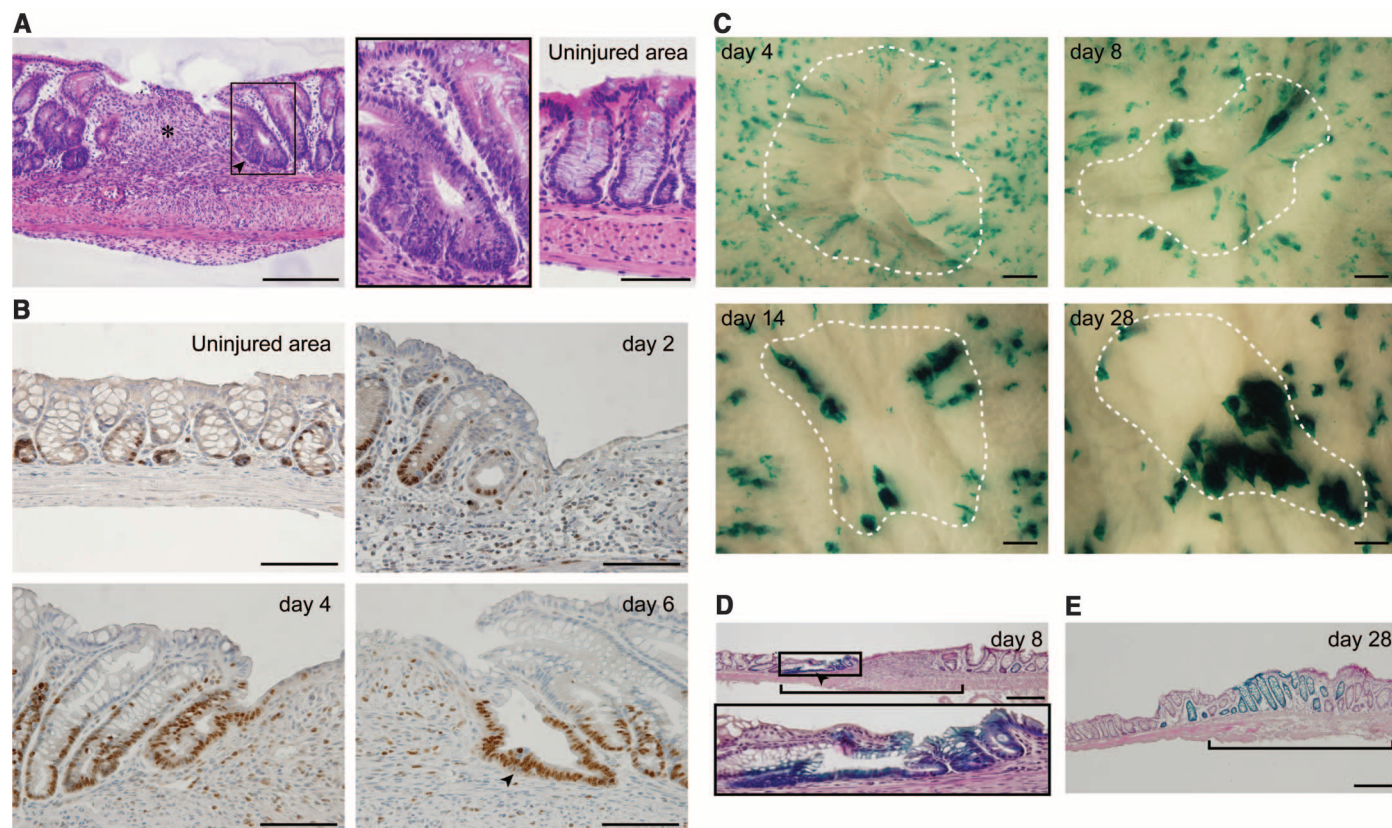


Fig. 1. Colonic crypts regenerate from existing crypts during injury repair. **(A)** A hematoxylin and eosin (H&E)-stained section of a wound at day 6 postinjury. The asterisk indicates the center of the wound bed; the arrowhead indicates a wound channel that consists of immature epithelial cells (boxed region, see inset). Crypts distant from the wound site are represented in the panel labeled “Uninjured area.” Scale bars, 200 μm . **(B)** Sections stained for Ki-67 (brown) to label proliferative cells at various time points after biopsy injury. An arrowhead labels the wound channel at day 6 postinjury. Scale bars, 100 μm . $n = 3$ wounds per time point for (A) and (B). **(C)** Migration of clonal cell populations from labeled crypts after

wounding in *Vil-CreERT: Rosa26R* mice. Cells expressing LacZ were visualized by the staining with 5-bromo-4-chloro-3-indolyl- β -D-galactoside. Dotted lines outline the original wound area. Scale bars, 200 μm . **(D)** A H&E-stained section of a wound at day 8 postinjury in a *Vil-CreERT: Rosa26R* mouse. LacZ-positive cells (blue) were present in a wound channel (arrowhead; see inset). The wound bed is delineated by a bracket. Scale bar, 200 μm . **(E)** A H&E-stained section of a wound at day 28 postinjury in a *Vil-CreERT: Rosa26R* mouse shows clusters of LacZ-positive crypts in the wound bed (shown by the bracket). Scale bar, 200 μm . $n = 6$ wounds per time point for (C), (D), and (E).

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transcription polymerase chain reaction (RT-PCR) (fig. S3C). Interestingly, mRNAs encoding the noncanonical Wnt ligand *Wnt5a* were significantly enriched in the wound bed compared with the adjacent uninjured mucosa (Fig. 2, A and B, and fig. S3, D and E).

Noncanonical Wnts expressed in undifferentiated mesenchymal cells affect tissue regeneration in hydra and zebrafish (16, 17). Similarly, in situ hybridization showed an expanded population of *Wnt5a*-positive stromal cells in the colonic wound bed (Fig. 2C and fig. S4) compared with uninjured mucosa (Fig. 2C) (18). Importantly, a subpopulation of *Wnt5a*-positive cells localized near wound channels. Similar to other

injury models, the *Wnt5a*-positive stromal cells in the colonic wound bed did not express markers of differentiation including α -smooth muscle actin (myofibroblasts) or β -catenin (endothelial cells) (fig. S5, A and B). We noted an additional subpopulation of *Wnt5a*-positive cells beneath the mucosal wound bed in the serosal area (fig. S6A). During development, one potential source of undifferentiated stromal progenitor cells in the mouse intestine is mesothelial cells that form the serosa (the outer surface of the intestinal tube) (19). We performed a genetic lineage tracing experiment for mesothelial cells using *WT1^{CreERT2}; Rosa26R* mice (20). Tamoxifen injection activates Cre recombinase in mesothelial cells that,

in turn, marks these cells by LacZ. A population of *Wnt5a*-positive cells was derived from these WT1-marked cells at day 6 postinjury (fig. S6, B to E), suggesting that *Wnt5a*-positive cells are partly derived from mesothelial cells.

We next examined the association of *Wnt5a*-positive cells with wound-channel epithelial cells. During injury repair, wound channels were composed of an expanded population of proliferative (Ki-67-positive), canonical Wnt-active (Axin2-positive) (21) epithelial cells. Notably, *Wnt5a*-positive cells localized to clefts at the base of wound channels, suggesting areas of nascent crypt formation (Fig. 2D). In addition, *Wnt5a*-positive cells were also located adjacent to

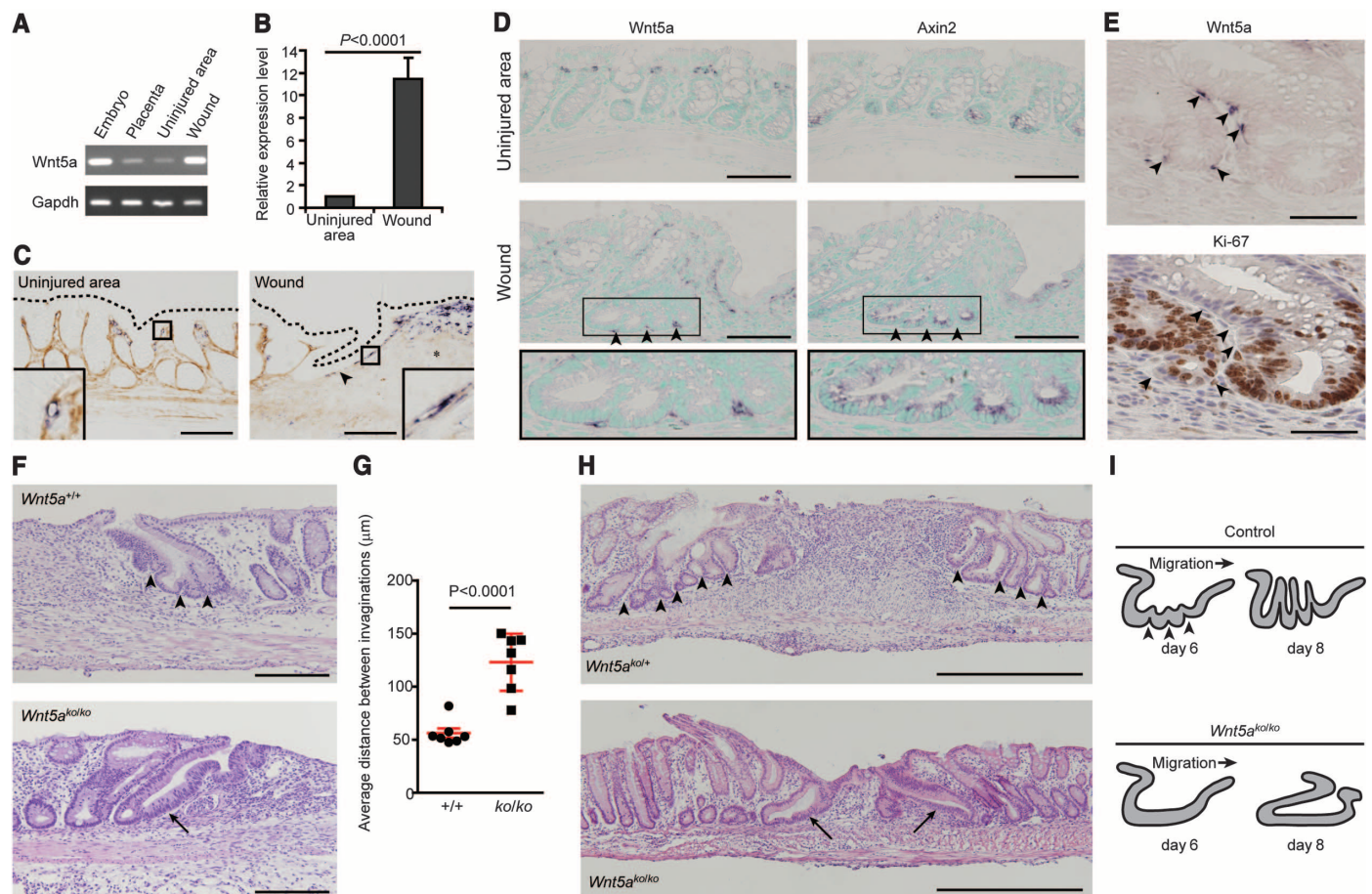


Fig. 2. *Wnt5a*-positive mesenchymal cells stimulate crypt regeneration after injury. (A) RT-PCR analysis of *Wnt5a* from RNAs isolated from an embryonic day 13.5 embryo and its placenta tissue (controls), the wound bed, and adjacent uninjured mucosa. *Gapdh*, glyceraldehyde-3-phosphate dehydrogenase. (B) Plots of mean ($\pm$ SD; error bar) relative *Wnt5a* mRNA expression levels as determined by quantitative RT-PCR analysis of wound beds and adjacent uninjured mucosa at day 4 postinjury. Data were analyzed using the Student's *t* test ($n = 4$ wounds per group). (C) Mouse colon sections at day 6 postinjury (uninjured area and wound) stained by in situ hybridization for *Wnt5a* (purple) and hyaluronic acid (basement membrane, brown). Dotted lines outline the apical epithelial surface. Scale bars, 100 μ m. (D) Serial sections of uninjured area and a colonic wound at day 6 postinjury stained for *Wnt5a* and Axin2 mRNA, respectively. Arrowheads indicate *Wnt5a*-positive cells associated with wound-channel clefts (insets). Methyl green labeled nuclei are shown. Scale bars, 100 μ m. (E) Serial sections of a colonic wound channel at day 6 postinjury stained for *Wnt5a* mRNA (top) and Ki-67 (bottom). *Wnt5a*-positive

cells (arrowheads) were localized near quiescent epithelial cells. Sections were counterstained with nuclear fast red (top) and hematoxylin (bottom). Scale bars, 100 μ m. $n = 3$ wounds per assay. (F) Sections from a *CAGGCreERTM;Wnt5a^{+/+}* (*Wnt5a^{+/+}*) mouse and a *CAGGCreERTM;Wnt5a^{flax/flax}* (*Wnt5a^{ko/ko}*) mouse at day 6 postinjury stained by H&E. Arrowheads indicate wound-channel invaginations. The arrow indicates an immature wound channel without invaginations. Scale bars, 200 μ m. (G) Graph of the average distance between wound-channel invaginations ($\pm$ SD) ($n = 7$ wounds per group). Each dot represents the average distance for an individual wound channel. Data were analyzed using Student's *t* test. (H) H&E-stained sections from a *Ubc-Cre-ER^{T2};Wnt5a^{flax/+}* (*Wnt5a^{ko/+}*) and *Ubc-Cre-ER^{T2};Wnt5a^{flax/flax}* (*Wnt5a^{ko/ko}*) mouse at day 8 postinjury ($n = 3$ mice analyzed per group). Arrowheads indicate the space between cryptlike structures that developed from wound channels. Arrows indicate abnormal immature wound channels with no cryptlike structures. Scale bars, 500 μ m. (I) Schematic diagrams of defects in crypt regeneration in *Wnt5a^{ko/ko}* mice.

nonproliferative wound-channel epithelial cells (Fig. 2E). This was specific for wound channels, as in uninjured areas, Wnt5a-positive mesenchymal cells were not associated with crypt bases that contain canonical Wnt-active epithelial cells (Fig. 2D). These results suggested that Wnt5a-positive mesenchymal cells may induce crypt formation by locally inhibiting proliferation of the stem/progenitor cell population within the wound channel.

To test this hypothesis in vivo, we generated a *Wnt5a* conditional knockout (ko) allele (fig. S7, A to D). We crossed *Wnt5a^{fllox}* mice with *CAGGCreERTM* (22) or *Ubc-Cre-ER^{T2}* (23) transgenic mice for global cellular targeting and generated CreERT-expressing *Wnt5a^{fllox/fllox}* mice (*Wnt5a^{ko/ko}*), as well as CreERT-expressing *Wnt5a^{fllox/+}* and *Wnt5a^{+/+}* mice (controls). After serial tamoxifen injections to activate Cre recombinase, we created colonic mucosal injuries and analyzed repair. *Wnt5a^{ko/ko}* mice generated by both methods contained normally appearing WAE cells (fig. S7E), but also contained abnormal wound channels at days 6 and 8 postinjury. Compared with uninjured controls, at day 6 postinjury, *Wnt5a^{ko/ko}* wound channels contained significantly fewer invaginations (Fig. 2, F and G), and at day 8 postinjury, they did not develop into new cryptlike structures (Fig. 2H). These results showed that Wnt5a has a crucial role in the proper formation and eventual subdivision of wound channels into crypts (Fig. 2I).

To test the direct effects of Wnt5a on wound-channel epithelium, we established an in vitro culture system that mimicked wound channels. Conditioned media from an L cell line express-

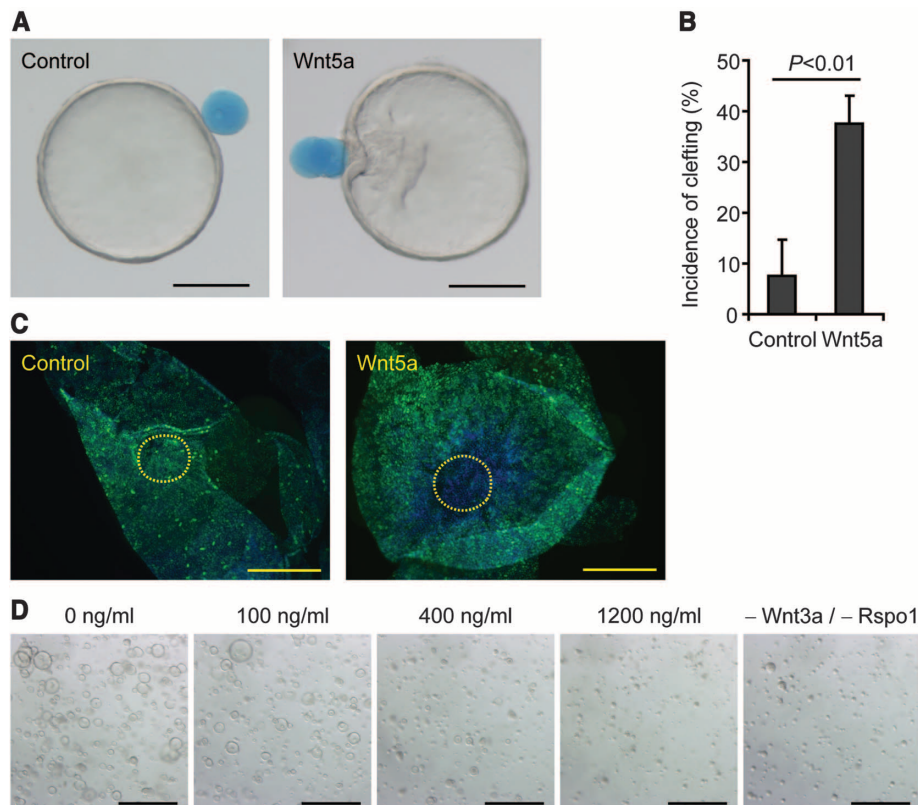
ing Wnt3a, R-spondin3, and noggin (L-WRN) (11, 13, 14) maintained highly proliferative, epithelial colonic stem/progenitor cells (Lgr5-positive) (24) as organoid spheres in culture (fig. S8 and tables S2 and S3); these properties were similar to in vivo wound-channel epithelial cells. Interestingly, Wnt5a-soaked beads (fig. S9) induced clefts within colonic organoids more frequently than control beads (Fig. 3, A and B). Epithelial proliferation in areas of contact with Wnt5a-soaked beads was suppressed compared with areas contacting control beads (Fig. 3C). Furthermore, addition of Wnt5a to the organoid culture medium suppressed colonic sphere formation stimulated by Wnt3a and R-spondin1 (Fig. 3D). Wnt5a decreased Ki-67 and Lgr5 expression in a dose-dependent manner (fig. S10A). However, Axin2 expression was not suppressed by Wnt5a (fig. S10A), in contrast to previous findings suggesting that Wnt5a antagonizes canonical Wnt signaling (25–28). The connection between noncanonical Wnt signaling and the cell cycle is unclear. Increased expression of cyclin-dependent kinase (Cdk) inhibitors can inhibit epithelial proliferation. We found that Wnt5a increased expression of multiple Cdk inhibitors, most notably p15^{INK4B} (Cdkn2b) in treated colonic epithelial organoids (fig. S10B). These results show that a focal source of noncanonical Wnt initiates a critical intermediary step to reestablish homeostasis of the colonic epithelium.

Because p15^{INK4B} expression can be induced by transforming growth factor- β (TGF- β) (29), a classical inhibitor of epithelial proliferation (30),

we tested whether Wnt5a activates the TGF- β signaling pathway. In colonic organoids, recombinant TGF- β 1 suppressed proliferation and induced Serpine1 (PAI-1) expression, indicating Smad transactivation (31) downstream of the TGF- β type 1 receptor (fig. S11, A and B). Surprisingly, Wnt5a also stimulated Serpine1 expression in a dose-dependent manner (Fig. 4A) and enhanced Smad3 phosphorylation/nuclear localization (Fig. 4B). In injured *Wnt5a^{ko/ko}* animals compared with control mice, the loss of Wnt5a expression was associated with diminished phosphorylation of Smad3 in the stem/progenitor population at the base of wound channels (Fig. 4, C and D, and fig. S11, C and D). The action of Wnt5a required kinase activity of the TGF- β receptor as SB431542, a kinase inhibitor for TGF- β type 1 receptor, suppressed all Wnt5a effects on cell growth, as well as Ki-67 and Serpine1 expression (Fig. 4E and fig. S11E). Thus, Wnt5a expression is required to potentiate TGF- β signaling at the base of wound channels and mimics the effects of Wnt5a in vitro.

Despite sharing several similar components, the canonical and noncanonical Wnt signaling pathways use distinct co-receptors, LRP5/6 and ROR1/2, respectively (32). Intestinal organoids exclusively expressed Ror2 (fig. S12), and *Ror2^{-/-}* and *Wnt5a^{-/-}* mice similarly display defective gut elongation (15, 33). Using colonic organoids with short hairpin RNA (shRNA) knockdown of Ror2 (fig. S13, A to C), we found that Wnt5a and TGF- β 1 showed diminished Serpine1 induction (Fig. 4F), indicating that the activation of the TGF- β signaling pathway by Wnt5a was

Fig. 3. Wnt5a inhibits proliferation of colonic epithelial stem cells. **(A)** Focal Wnt5a-induced clefts in colonic epithelial organoids. A control (left) or a Wnt5a-soaked bead (right) was placed adjacent to different colonic organoids. Scale bars, 200 μ m. **(B)** Plot of the mean cleft incidence of colonic organoids ($\pm$ SD; error bars) attached to control or Wnt5a-soaked beads ($n = 3$ experiments). A Student's *t* test was used to determine significance. **(C)** Colonic organoids attached to either control (left) or Wnt5a-soaked beads (right) were stained for Ki-67 (green). Yellow dotted lines outline the bead attachment area. Nuclei were counterstained with bis-benzimide (blue). Representative images from three samples (per group) are shown. Scale bars, 200 μ m. **(D)** Representative images of colonic epithelial organoids cultured for 48 hours in Wnt3a/Rspo1 and indicated amounts of Wnt5a. The rightmost panel is a control without Wnt3a/Rspo1 ($n = 3$ experiments). Scale bars, 500 μ m.



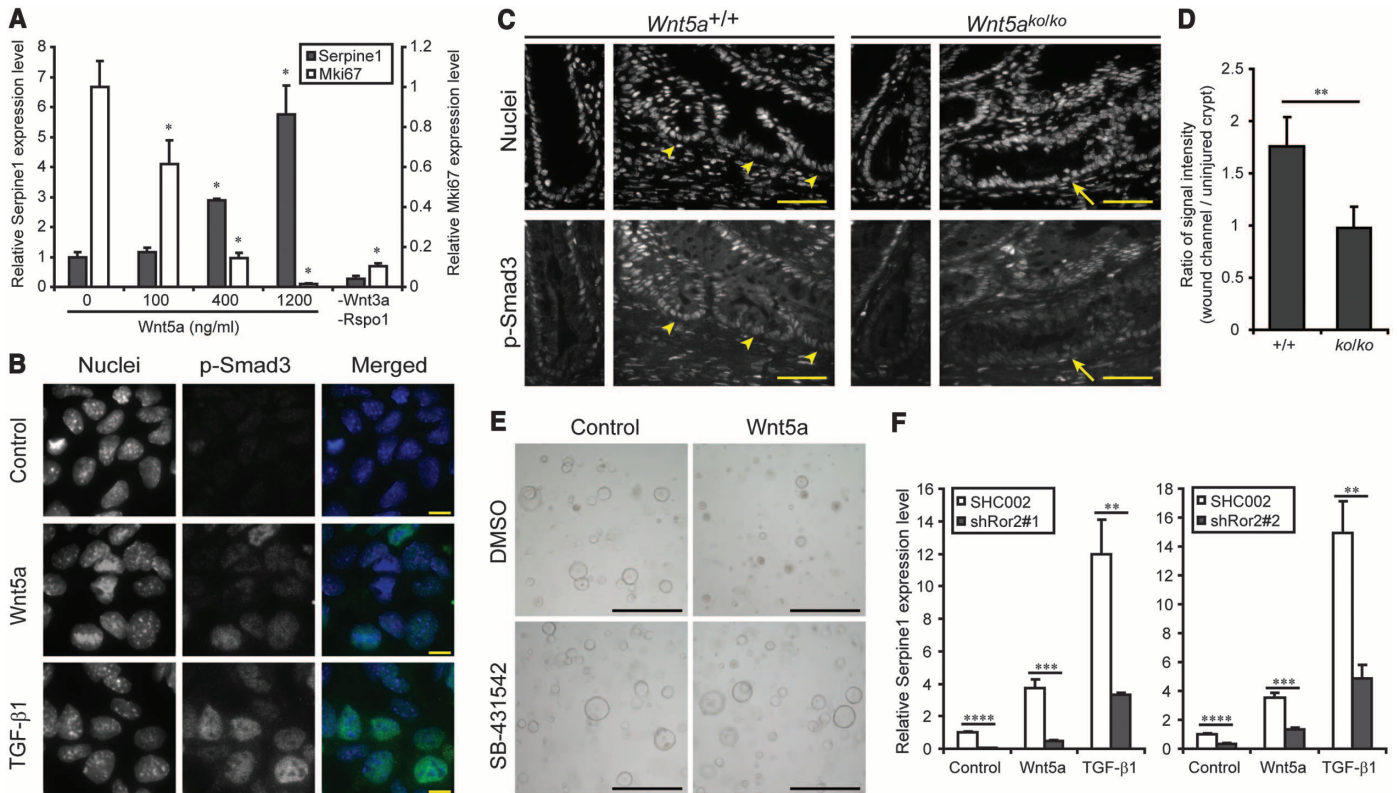


Fig. 4. Wnt5a activates the TGF- β signaling pathway. **(A)** Colonic organoids were cultured for 24 hours with recombinant Wnt5a. Plots of mean ($\pm$ SD; error bars) relative mRNA expression levels of Serpine1 and Mki67 were determined by quantitative RT-PCR analysis ($n = 3$ samples per group). Data were analyzed using one-way analysis of variance followed by Tukey's test ($P < 0.0001$). Asterisks indicate differences, compared with the baseline condition (0 ng/ml), that were significant ($P < 0.05$) in the posttest. **(B)** Nuclear localization of p-Smad3 protein. Colonic epithelial cells grown on Matrigel-coated chambers (Matrigel, BD, Franklin Lakes, NJ) were incubated without ligands (control) and with either Wnt5a (400 ng/ml) or TGF- β 1 (1 ng/ml) for 2 hours and then fixed and stained for p-Smad3 (representative images from three experiments). Scale bars, 10 μ m. **(C)** Distribution of p-Smad3 in the wound channels (right) and uninjured crypt units (left). Colonic sections from *Ubc-Cre-ER^{T2}; Wnt5a^{+/+}* (*Wnt5a^{+/+}*) and *Ubc-Cre-ER^{T2}; Wnt5a^{lox/lox}* (*Wnt5a^{ko/ko}*) wounds at day 6 postinjury were stained for p-Smad3 (bottom). Cell nuclei were visualized with bis-benzimide (top).

Arrowheads and arrows indicate the base of wound channels. Scale bars, 50 μ m. **(D)** Quantification of p-Smad3 in wound channels. Plots of the mean ratio of signal intensity ($\pm$ SD) in epithelial cells located in the base of wound channels compared with the base of crypts in unwounded areas (from the same tissue section) were determined as described in the supplemental materials and methods ($n = 4$ wounds per group). **(E)** Colonic organoids were cultured for 24 hours without ligands (control) and with Wnt5a (400 ng/ml), SB-431542 (10 μ M), or both together. Representative bright-field pictures were shown ($n = 3$ experiments). DMSO, dimethyl sulfoxide. Scale bars, 500 μ m. **(F)** Colonic organoids were cultured for 24 hours without ligands (control) and with Wnt5a (400 ng/ml) and TGF- β 1 (1 ng/ml). Two pairs of populations expressing shRNA for independent target sequences and their controls (SHC002) were examined. Plots of mean ($\pm$ SD) relative mRNA expression levels were determined by quantitative RT-PCR analysis ($n = 3$ samples per group). The asterisks in (D) and (F) indicate differences that were significant in the Student's t test: ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

mediated through Ror2. Ror2 knockdown did not affect growth inhibition of Wnt5a and TGF- β 1 (fig. S14), suggesting that TGF- β inhibited the cell cycle in a transcription-independent manner, as previously described (34), and that the signaling through Ror1/2 enhanced transcriptional activity of the Smad protein complex. Although the core components of the TGF- β signaling pathway consisting of type I and II receptors and Smad proteins are relatively simple, regulatory mechanisms (including protein modification and translocation) are highly complex (35). This experimental system will help to identify more precise molecular mechanisms of cell cycle inhibition by noncanonical Wnt and TGF- β .

Here, we show that wound channels are a critical intermediate structure during colonic epithelial wound repair. These channels undergo Wnt5a-mediated subdivision via a previously un-

known mechanism to potentiate TGF- β signaling. The effects of Wnt5a are primarily mediated by focal inhibition of proliferation, though we cannot rule out effects on cell polarity/asymmetric cell division. The process of crypt regeneration appears to reuse elements of a fetal developmental program (15, 36). Even though a colonic mesenchymal niche is not yet defined, our findings suggest that Wnt5a-positive cells are a critical part of this niche during injury repair, as they affect the organization of the regenerating epithelium. We propose that such epithelial-mesenchymal interactions during repair will occur in other tissues and organisms (1), such as hair follicles, that elevate specific Wnt family members during regeneration (37).

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Materials transfer agreements will be required for the acquisition of the *Wnt5a*^{fl} mice from the National Cancer Institute and the L-WRN cell line from the Washington Univ. Medical School. The data presented in this manuscript are tabulated in the main paper and in the supplementary materials.

Supplementary Materials

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Materials and Methods

Figs. S1 to S14
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References (38–53)

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Insect Herbivores Drive Real-Time Ecological and Evolutionary Change in Plant Populations

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Insect herbivores are hypothesized to be major factors affecting the ecology and evolution of plants. We tested this prediction by suppressing insects in replicated field populations of a native plant, *Oenothera biennis*, which reduced seed predation, altered interspecific competitive dynamics, and resulted in rapid evolutionary divergence. Comparative genotyping and phenotyping of nearly 12,000 *O. biennis* individuals revealed that in plots protected from insects, resistance to herbivores declined through time owing to changes in flowering time and lower defensive ellagitannins in fruits, whereas plant competitive ability increased. This independent real-time evolution of plant resistance and competitive ability in the field resulted from the relaxation of direct selective effects of insects on plant defense and through indirect effects due to reduced herbivory on plant competitors.

The ubiquitous consumption of plants by insect herbivores represents one of the dominant species interactions on Earth and has been hypothesized to play a strong role in the diversification of plant species and their traits (1–3). The evolution of plant defense has been studied primarily with either a prospective or retrospective approach. Single-generation prospective approaches measure contemporary natural selection on resistance traits and make predictions about how those traits should evolve given estimates of their heritability (4–6). By contrast, retrospective studies compare populations or species that have diverged over time (7–9) and make inferences about the processes driving evolutionary change. More generally, temporal studies of natural species interactions that influence evolutionary dynamics remain rare, and few have been exper-

imental (9–12). Thus, we lack experimental field studies that quantify how species interactions influence selection on traits and the evolutionary response to this selection. As such, it is not well

established how rapidly plants adapt to selection by herbivores, which traits are most important in the evolution of defense, and whether herbivory drives predictable parallel changes due to selection across populations.

The ecology and evolution of plant-herbivore relationships is, in part, governed by the reciprocal nature of their interaction and the complexity of communities. For example, plant traits and plant community structure are critical for determining insect occurrence and attack rates (1–3). Conversely, insects can directly reduce focal plant abundance (13) or indirectly affect plant abundance through changes in the density of co-occurring competitors (14). Thus, the evolutionary dynamics of a focal plant species might be influenced through direct selection on resistance traits and/or by indirect selection on traits influencing competitive ability (15, 16). Despite this likely scenario, our understanding of how species interactions influence the evolution of constituent community members is surprisingly limited (17).

We conducted a field study of the selective impact of insects and the evolutionary response

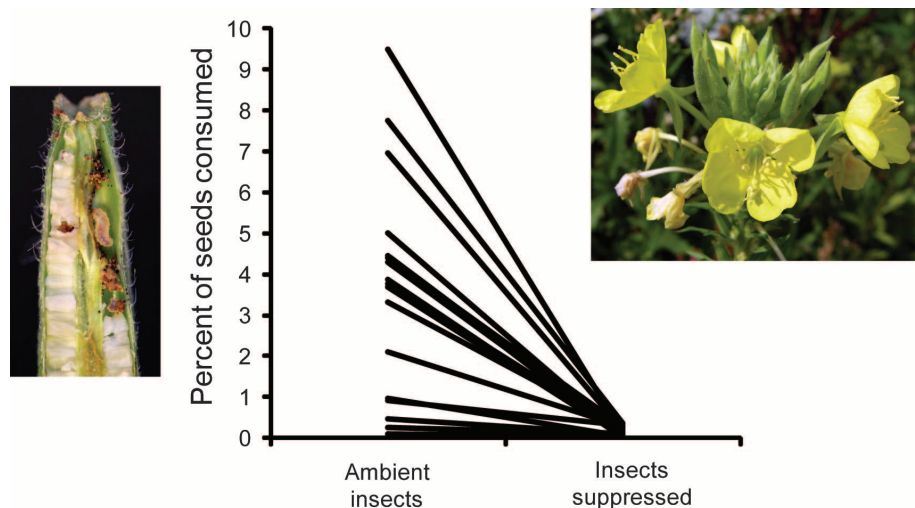


Fig. 1. Fruit loss to seed predator moths (dominated by *M. brevivittella*) in 18 genotypes of *O. biennis* in ambient and insect-suppressed plots (data are from the first generation, 2007–2008, mixed-model analysis of variance, genotype-by-insecticide interaction: $\chi^2 = 57.55$, $P < 0.001$). (Left) an opened fruit with a *M. brevivittella* larvae consuming immature seeds; (right) a flowering *O. biennis*.

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to this selection in the native forb common evening primrose, *Oenothera biennis* (Onagraceae) (18). We established 16 replicate plots planted with 60 individual seedlings of *O. biennis*, containing equivalent frequencies of 18 uniquely identified genotypes (19). Half of the plots were treated biweekly during each growing season with esfenvalerate, a nonsystemic insecticide (insect suppression treatment, supplementary text 2a); the remaining plots received an equivalent amount of water as a control (ambient insect treatment). Because *O. biennis* is annual or biennial, primarily selfing, and has a genetic system that suppresses recombination and segregation of alleles, we could track changes in frequencies of each planted genotype within each replicate population over multiple generations (19). Plots were not further weeded or otherwise manipulated, allowing the natural assembly and early succession of plant species with and without insects (supplementary text 2b). We previously summarized our sampling methods, the effects of flowering phenology on insect attack, and plant life-history evolution in the ambient insect plots (19). Here, we address the effects of experimental insect suppression on ecological and evolutionary change in *O. biennis*, including changes in genotypic composition, defensive chemistry, competitive ability, and flowering time.

Although there were three native species of specialist seed predator moths at our site, fruit

loss in *O. biennis* was dominated by *Mompha breviovittella*, which was >5 times as abundant as *M. stellerella* and *Schinia florida* combined, the other two common specialists. The 18 *O. biennis* genotypes varied substantially in their resistance to these seed predators (Fig. 1), and insect suppression created an environment with potentially large differences in natural selection. Despite increases in *O. biennis* abundance in the first 2 years of the experiment (reaching up to 4000 individuals per plot in 2009), recruitment was substantially lower in the insect suppression plots compared to ambient controls (Fig. 2). This reduction in *O. biennis* density coincided with a rise in the abundance of common dandelion, *Taraxacum officinale* (Asteraceae), an early successional weed that dominated our plots and was twice as abundant in plots where insects were suppressed compared to controls (Fig. 2 and fig. S1).

A specialist seed-feeding beetle of *T. officinale*, *Glocianus punctiger*, was significantly reduced by insect suppression, and a highly abundant generalist moth caterpillar at the site, *Noctua pronuba*, showed a preference for *T. officinale* over *O. biennis* (supplementary text 2c). Thus, we hypothesized that the release of *T. officinale* from its herbivores led to competitive suppression of *O. biennis*. Indeed, populations of *O. biennis* and *T. officinale* showed a negative correlation at the population level (simple correlation, $N = 16$, $r = -0.523$, $P =$

0.038), and an independent field experiment at the same site confirmed that *T. officinale* inhibits early establishment of *O. biennis* (supplementary text 2d). Because *O. biennis* requires light for germination, we hypothesize that *T. officinale* likely affects germination as well as early seedling survival. Therefore, insect suppression substantially affected plant competitors in addition to our focal species.

The net evolutionary consequence of insect suppression was a change in the genetic structure of *O. biennis* populations (Fig. 3, repeated measures canonical correspondence analysis, permutation test, trace = 0.275, $F = 8.511$, $P = 0.020$), with the strongest effect observed in the final year of the experiment (fig. S2). This parallel genetic differentiation among our replicated insect suppression plots in comparison to control plots (Fig. 3) implies that selection by insects, and not genetic drift, drove the observed

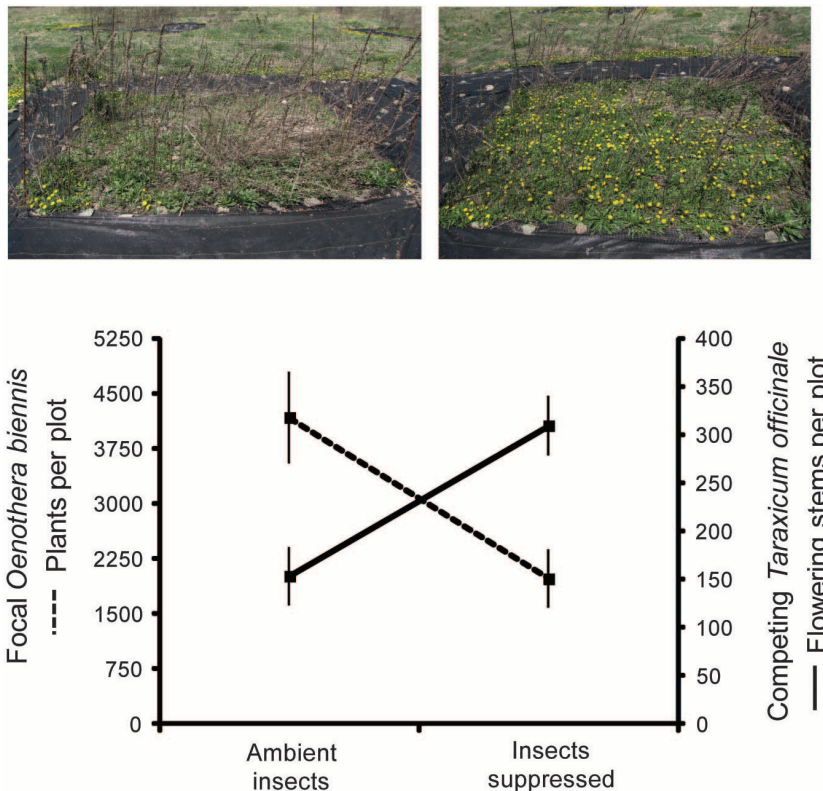


Fig. 2. Effects of insect suppression on the densities of focal *O. biennis* (univariate analysis of variance, $F_{1,14} = 8.97$, $P = 0.010$) and a major colonizing competitor (*T. officinale*, univariate analysis of variance, $F_{1,14} = 12.96$, $P = 0.003$) in 2009 (means $\pm$ SEM); also shown are representative ambient and insect-suppressed plots when *T. officinale* was in peak bloom.

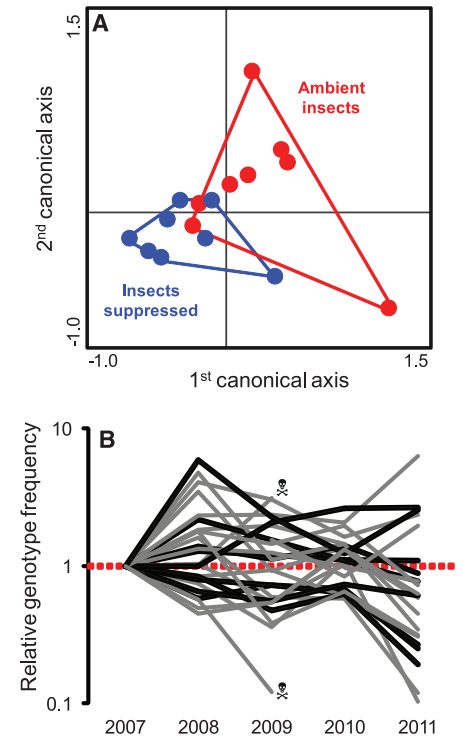


Fig. 3. (A) Results from an unconstrained correspondence analysis on frequencies of all genotypes in 2011. Each dot represents an experimental plot; ambient and suppressed treatments were coded after the analysis. A constrained canonical correspondence analysis revealed substantial genetic differentiation between treatments (data from 2011, permutation test, trace = 0.127, $F = 2.211$, $P = 0.003$). (B) The relative frequency (ambient/suppressed) of each genotype over time. The dashed red line indicates the null hypothesis of no divergence in genotype frequencies between treatments; darker lines highlight genotypes that represented >2% of the plants in 2011. Skull and crossbones indicate two genotypes that went extinct in one treatment. The y axis is on a logarithmic scale to facilitate equal visualization of under- and overrepresented genotype frequencies.

genotypic evolution. At the end of the experiment, 1 of the original 18 genotypes was entirely extirpated, and two additional genotypes were represented only in insect suppression plots (Fig. 3). Only six genotypes consistently maintained a frequency of 2% or higher within the populations. Three novel genotypes, derived from outcrossing events between known parents, each also reached >2% of the populations in 2011 (supplementary text 2e). Thus, across both treatments, nine genotypes dominated all plots (>2% each) at the end of the experiment. Genotypic evenness was >50% higher in insect suppression plots compared to controls (Smith and Wilson's evenness index, E_{var} , univariate analysis of variance, $F_{1,14} = 15.037$, $P = 0.002$), suggesting an erosion of genotypic variance in the presence of insect herbivores. Nonetheless, insect suppression affected neither genotypic richness (univariate analysis of variance, $F_{1,14} = 0.287$, $P = 0.600$) nor diversity (Simpson's index, univariate analysis of variance, $F_{1,14} = 0.377$, $P = 0.549$).

On the basis of annual estimates of genotype frequencies in each plot and genotype-specific means of *M. brevivittella* attack measured in control plots during 2007, we found that experimental suppression of insects resulted in evolution of relaxed plant defense (Fig. 4). We next determined the evolutionary response of traits responsible for this divergence in resistance. We had previously shown that the extent of early-season flowering (number of open or senesced

flowers after 50% of the plants had at least a single open flower) positively correlated with *M. brevivittella* attack in 2 years (19) (i.e., later-flowering plants suffered less damage). There was no difference between treatments in the first year of our study (2007), as early season flowering was not different among the two treatments (univariate analysis of variance, $F_{1,14} = 0.320$, $P = 0.581$); by 2010, there was a significant shift toward earlier flowering in plots with suppressed insects (univariate analysis of variance, $F_{1,14} = 6.105$, $P = 0.027$). This effect was even stronger in 2011 (Fig. 4, univariate analysis of variance, $F_{1,14} = 7.025$, $P = 0.019$) and was predictable on the basis of genotype frequencies and genotype-specific means for early flowering (Fig. 4). Thus, a predicted response to selection was evident in only three to four generations. We found no difference in annual versus biennial phenotypes of *O. biennis* (repeated measures multivariate analysis of variance, $F_{1,14} = 0.935$, $P = 0.350$).

O. biennis produces diverse hydrolyzable tannins, including the largest ellagitannins known from any plant species (20), and these compounds have very high oxidative capacity, negatively affecting insect herbivores (21). One trimer, oenothain A (fig. S3), was the dominant ellagitannin in *O. biennis* fruits (up to 7.8% dry mass). We previously showed that oenothain A is favored by natural selection in *O. biennis* leaves (6), and its production in fruits was negatively genetically correlated with *M. brevivittella* attack in

the current study (simple correlation, $N = 17$, $r = -0.491$, $P = 0.045$) (supplementary text 2f), implicating a role of this compound in defense. Despite a genetic correlation between foliar and fruit ellagitannin chemistry (simple correlation, $N = 18$, $r = 0.848$, $P < 0.001$), only fruit chemistry significantly diverged in our experimental plots (Fig. 4), suggesting that selection was strongest for defense against flower- and fruit-feeding herbivores like *M. brevivittella*. A negative genetic correlation between early-season flowering and the production of oenothain A in fruits (simple correlation, $N = 23$, $r = -0.67$, $P < 0.001$) is consistent with the rapid and joint evolution of these traits (i.e., early flowering with low oenothain A in insect-suppressed plots) (Fig. 4).

Given that insect suppression not only affected herbivory on *O. biennis*, but also resulted in stronger plant competition through larger *T. officinale* populations, we hypothesized that *O. biennis* would evolve greater competitive ability in the absence of insects. We measured the relative competition index (RCI) in total above- and below-ground biomass of different genotypes in an independent experiment (fig. S4). We then used these genotype-specific values of competitive ability to determine if the competitive phenotypes diverged between control and insect-suppressed plots. Insect suppression resulted in the evolution of enhanced competitive ability relative to ambient insect plots (Fig. 4), and because this effect was not genetically correlated with early-season

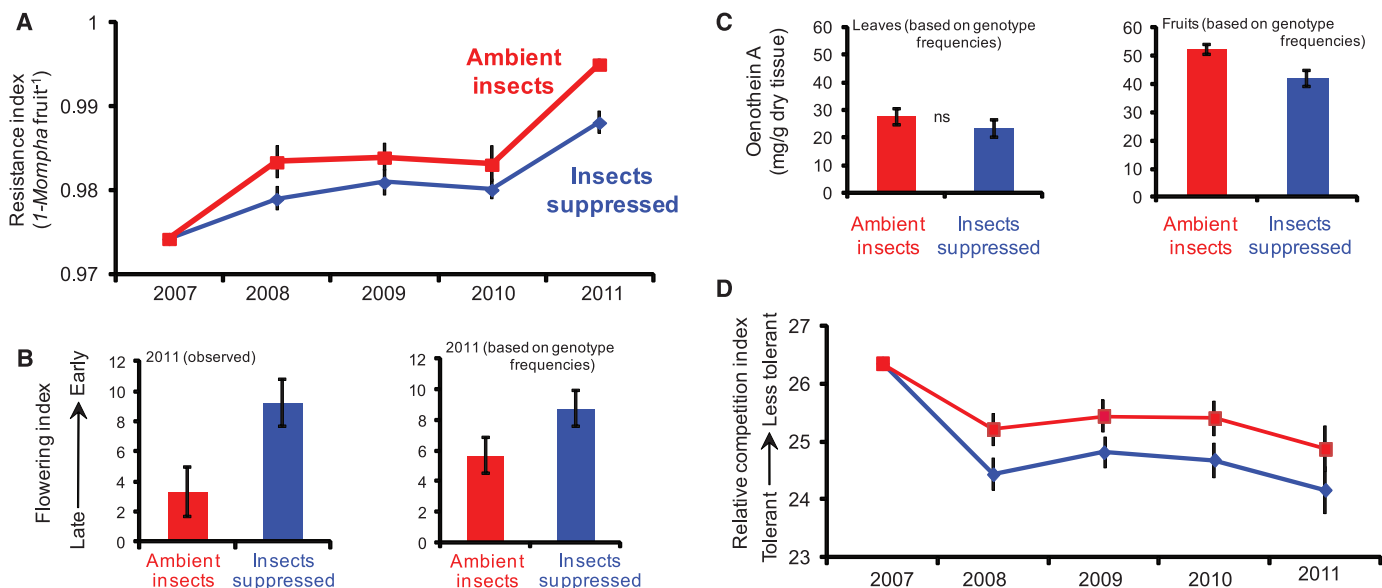


Fig. 4. Phenotypic evolution in *O. biennis* plots. **(A)** In the presence of seed predator insects, higher plant resistance evolved [estimated by multiplying genotype-specific means measured in control (ambient insect) plots by genotype frequencies in each plot] (repeated measures multivariate analysis of variance, $F_{1,14} = 6.322$, $P = 0.025$), and this effect did not vary over time (time-by-treatment interaction, repeated measures multivariate analysis of variance, $F_{3,12} = 1.459$, $P = 0.275$). **(B)** Earlier flowering was observed in the insect suppression plots (univariate analysis of variance, $F_{1,14} = 7.025$, $P = 0.019$). We confirmed that this was an evolutionary response by multiplying genotype-specific means by genotype frequencies (univariate analysis of variance, $F_{1,14} = 4.530$, $P = 0.050$); the former field assessment includes the possible effects of

phenotypic plasticity, whereas the latter is based on genotypic values. **(C)** Leaf defensive chemistry did not diverge (univariate analysis of variance, $F_{1,14} = 1.716$, $P = 0.211$) but fruit chemistry changed (univariate analysis of variance, $F_{1,14} = 8.917$, $P = 0.010$) as predicted by genotypic means and genotype frequencies. **(D)** Competitive ability of *O. biennis* when grown with *T. officinale*; plants in the insect-suppressed plots evolved a greater ability to maintain above- and belowground biomass (relative competition index) when grown with a competitor than when grown alone (repeated measures multivariate analysis of variance, $F_{1,14} = 5.189$, $P = 0.038$); this effect did not vary over time (repeated measures multivariate analysis of variance, time-by-treatment interaction ($F_{3,12} = 0.072$, $P = 0.974$)). All panels show means $\pm$ SEM; ns, not significant.

flowering (simple correlation, $N = 23$, $r = -0.157$, $P = 0.474$), the production of oenothien A (simple correlation, $N = 23$, $r = 0.035$, $P = 0.871$), or resistance to *M. brevivittella* (simple correlation, $N = 17$, $r = -0.255$, $P = 0.241$), responses to natural selection were unconstrained by genetic correlations. The evolution of increased susceptibility to herbivores and enhanced competitive ability was jointly favored by the suppression of insects.

Concurrent evolution of herbivore resistance and competitive ability was only partially predictable from annual measures of natural selection. We correlated trait values against lifetime seed production in each year, hypothesizing that we would observe divergent slopes concordant with the divergent evolutionary change that we observed between our two treatments. Although we found significantly divergent natural selection between ambient and insect suppression plots for the two resistance traits, this effect was observed in only 1 of 4 years (fig. S5). Nonetheless, when differences in natural selection were observed, they were in the predicted direction on the basis of the observed evolutionary change (table S1). Thus, annual measures of natural selection predicted from fecundity were not strong predictors of evolutionary change, which suggests that selection on other components of fitness contribute to observed evolutionary responses (19).

Our study provides definitive evidence for the rapid evolution of insect-mediated plant resistance traits in real time, supporting current interest in reciprocal feedbacks between evolutionary change and ecological dynamics (17, 19, 22, 23). We highlight how biotic environmental factors simultaneously alter ecological and evolutionary dynamics. Specifically, our finding that multiple

herbivores attacking co-occurring host plants can alter competitive interactions is likely quite general in natural ecosystems (14, 24, 25), and the rapid evolutionary responses observed confirm predicted interactive effects of herbivory and competition on plant evolution (15, 16). Given that *O. biennis* is a poor competitor, we predict that evolutionary shifts in resistance and competitive ability allow this species to persist for longer periods of time during succession.

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Supplementary Materials

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Materials and Methods
Supplementary Text
Figs. S1 to S5
Table S1
References (26–32)

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Natural Enemies Drive Geographic Variation in Plant Defenses

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Plants defend themselves against attack by natural enemies, and these defenses vary widely across populations. However, whether communities of natural enemies are a sufficiently potent force to maintain polymorphisms in defensive traits is largely unknown. Here, we exploit the genetic resources of *Arabidopsis thaliana*, coupled with 39 years of field data on aphid abundance, to (i) demonstrate that geographic patterns in a polymorphic defense locus (*GS-ELONG*) are strongly correlated with changes in the relative abundance of two specialist aphids; and (ii) demonstrate differential selection by the two aphids on *GS-ELONG*, using a multigeneration selection experiment. We thereby show a causal link between variation in abundance of the two specialist aphids and the geographic pattern at *GS-ELONG*, which highlights the potency of natural enemies as selective forces.

Intraspecific genetic variation is essential in enabling species to respond rapidly to evolutionary challenges such as changing environ-

mental conditions (1) or the emergence of novel pests and pathogens (2). This diversity often reflects the balance between the strength of local

selection and the current and historical levels of population substructure and gene flow (3, 4). Geographic analyses of genetic variation in several plant species have revealed clear genetic signals of local adaptation (5), caused by differences in the selective regime among locations. These analyses are further supported by reciprocal transplant experiments, in which home genotypes generally outperform those transplanted from other populations (6, 7). Although the drivers of local adaptation often remain unidentified, there

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is evidence that climate and soil can exert strong local selective pressures and play important roles in shaping large-scale genetic patterns (8, 9).

In contrast to the clear role of abiotic factors, there is little direct evidence that biotic forces, such as herbivory or competition, can lead to the maintenance of genetic variation across large geographic scales, despite the exceptional levels of polymorphism associated with genes involved in defense (10, 11). In theory, interactions between organisms and their natural enemies can lead to differences in the local selective regime because of geographic variation in the abundance or species composition of the enemy community (3). This spatial variation can affect defense if it is costly; e.g., if the average level of herbivory varies across populations, defended genotypes might dominate in heavily attacked populations whereas undefended genotypes would prosper when enemies are absent or rare (12). Another less studied effect is how defense might vary if plants are attacked by diverse collections of herbivore species that differ in feeding style and specialization. This could lead to higher levels of polymorphism in defense genes due to selection for specific defensive profiles matched to the predominant local herbivore or herbivore community [e.g., (13)]. However, there is no direct evidence that variation in local herbivore communities represents a sufficiently strong selective pressure to favor specific defensive traits and maintain polymorphisms in defense-related genes.

The unparalleled genetic and molecular resources available for the model plant *Arabidopsis thaliana* make this species an ideal candidate to

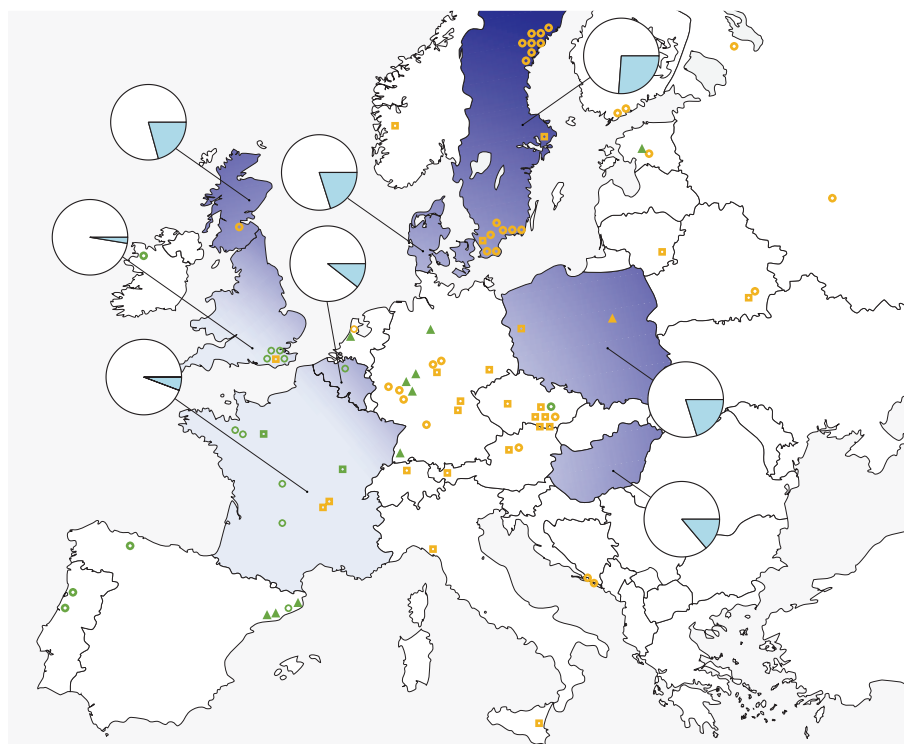
study the process of local adaptation to herbivores. The primary defensive trait in *A. thaliana* is a series of indolic and aliphatic glucosinolates, which are secondary plant metabolites with antiherbivore properties (14). The accumulation and structure of aliphatic glucosinolates are mechanistically determined by alleles at the *GS-ELONG* locus that regulate the carbon side-chain elongation (3C or 4C) (15) and by alleles at the *GS-AOP* locus that modify the functional group of the biologically active glucosinolate side chain (*ALK*, *OH*, or *NULL*). The combination of these alleles yields six distinct chemotypes present in natural populations in varying proportions (16). Both individual glucosinolate compounds and full chemical profiles affect the susceptibility of a plant to specific herbivores (17, 18); hence, the aliphatic chemotype is likely under differential, qualitative selection by herbivores. By contrast, accumulation of the main indolic glucosinolates in *A. thaliana* is highly plastic and modulated by a large number of small-effect genetic loci, which are therefore less likely to show clear signatures of selection (19).

We mapped geographic variation in the abundance of the six chemotypes within Europe from a set of 96 accessions (75 European) (20) with known chemical profiles (16) (Fig. 1). There was no apparent pattern in the distribution of the *GS-AOP* chemotypes, but for *GS-ELONG*, the frequency of 3C to 4C chemotypes increases with both latitude and longitude (Fig. 1 and fig. S1). If this pattern results from geographical variation in herbivore feeding pressure, we would expect it to be closely matched by variation in herbivore abundance patterns. Although *A. thaliana*

is attacked by a range of invertebrate herbivores, many of which preferentially feed on specific chemotypes (17), we hypothesized that the aphid species *Brevicoryne brassicae* and *Lipaphis erysimi* are likely drivers of these patterns as they are both abundant, mobile Brassicaceae specialists, yet differentially sensitive to environmental conditions (21). Fluctuations in aphid populations have been monitored since 1964 through the EXAMINE network (www.rothamsted.ac.uk/examine/) with suction traps that operate throughout the aphid flight season (22). We retrieved data on the two aphid species from 61 traps in eight European countries. These data revealed that the abundance of *L. erysimi* is usually lower than that of *B. brassicae*, but that the geographic pattern in the relative abundances of *L. erysimi* and *B. brassicae* closely mirrors the pattern at *GS-ELONG* (Fig. 1 and fig. S1). Variation in the relative abundance of these two specialist aphids could therefore underlie variation in the predominant *GS-ELONG* chemotype found in natural populations.

Because causal inferences are impossible from such correlative data, we tested the causality of aphid selection on *GS-ELONG*, carrying out a multigenerational selection experiment on populations of *A. thaliana* (22). We assembled 30 replicate populations from equal numbers of seeds from each of 27 natural accessions, including 6 of the 75 European accessions mapped above. Accessions were chosen to maximize variation in defense traits while including all six glucosinolate chemotypes in a range of genetic backgrounds (table S1 and fig. S2). Over five generations, we consistently exposed populations to replicate

Fig. 1. Location of European *A. thaliana* accessions with known chemical profile. Symbol color indicates the *GS-ELONG* chemotype (orange: 3C; green: 4C) and symbol shape indicates the *GS-AOP* chemotype (square: *ALK*; circle: *OH*; triangle: *NULL*). For *GS-ELONG*, the probability of finding 3C populations increases strongly with longitude (binomial GLM: $t = 5.11$, $df = 85$, $P < 0.001$) and weakly with latitude ($t = 1.75$, $df = 85$, $P = 0.084$). Countries with available aphid data are colored in blue. The shade of blue corresponds to the relative frequency of *L. erysimi* based on model predictions from a binomial GLM using data from 61 aphid suction traps. The relative frequency of *L. erysimi* increases strongly with longitude ($t = 5.03$, $P < 0.001$) and weakly with latitude ($t = 1.89$, $P = 0.060$). Pie charts indicate the observed average relative abundance of *B. brassicae* (white) and *L. erysimi* (blue) in each country.



(*n* = 6) treatments of a single specialist aphid species: either *B. brassicae* or *L. erysimi*; a single generalist aphid, *Myzus persicae*; a mixture of all three aphid species; and a no-aphid treatment. The generalist aphid was included as a negative control, because *M. persicae* is unresponsive to aliphatic glucosinolates (23) and we therefore would not expect it to exert directional selection on plant

chemotype. The no-aphid treatment served as a control for other selective forces that were likely to affect the outcome of the experiment, such as intraspecific competition among accessions. Seeds were collected at the end of each generation with no mixing among populations, and a subset was used to establish the next generation at a constant density. After five generations of repeated herbi-

vore treatments, we sampled 24 individuals from each population in generation 5 (144 individuals per treatment) and determined their genotype. To have a marker for changes in genotypic composition through time, we also measured leaf trichome density, a trait under strong genetic control (fig. S3), on a representative sample of plants in all generations.

Fig. 2. (A) Change in the negative impact of aphid treatments on final plant biomass over five generations, displayed as the log-difference to final plant biomass in the no-aphid treatment (dashed line): *B. brassicae* (light blue); *M. persicae* (light green); *L. erysimi* (orange); and aphid mixture (yellow). Stars denote significantly less damage after five generations of selection (table S2). (B) Mean number of trichomes on the fourth leaf of 50 plants per population. Stars denote significant difference from the no-aphid treatment (black line) after five generations of selection.

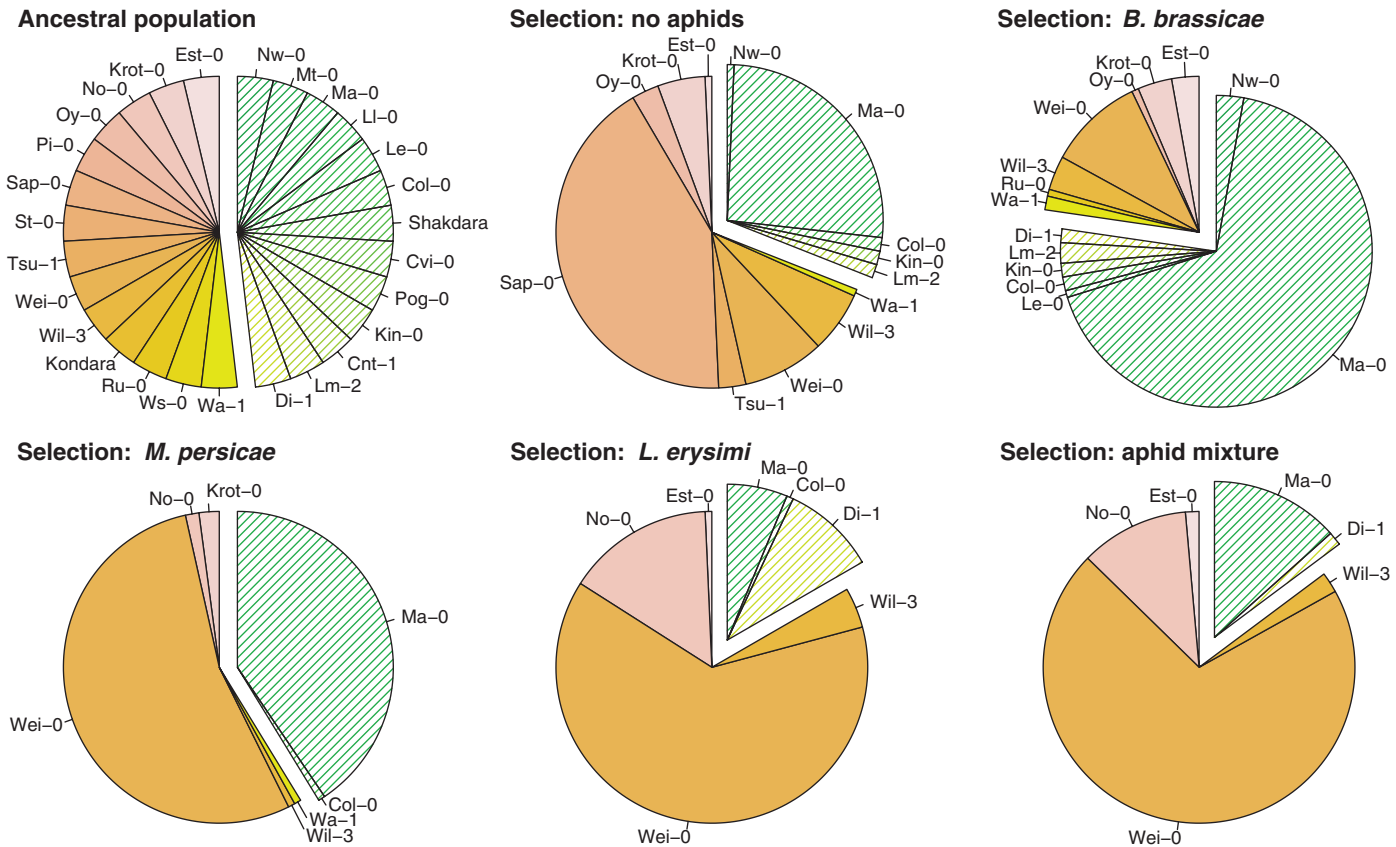
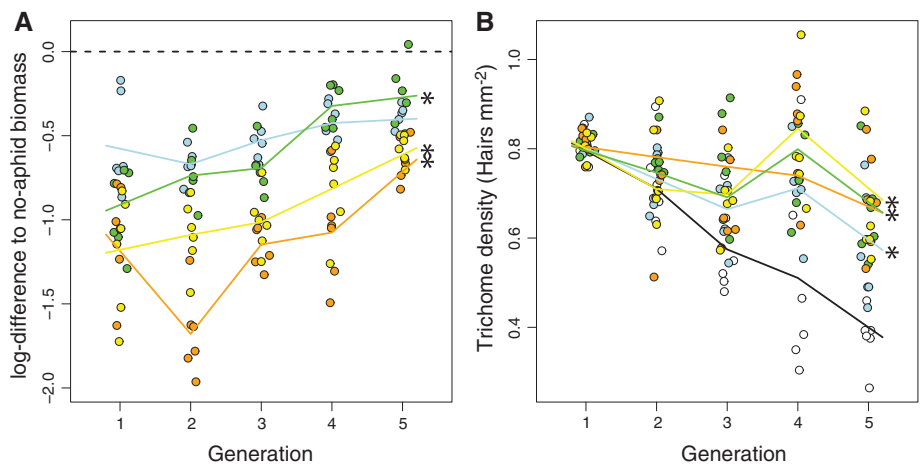


Fig. 3. Change in the composition of *A. thaliana* accessions, from equal proportions of 27 genotypes in the ancestral population to treatment-specific compositions after five generations of selection. Each chart gives mean genotype frequencies based on *n* = 6 replicate populations. 3C chemotypes are indicated by solid, orange colors, and 4C chemotypes by hatched, green colors.

Rapid adaptation occurred in the selection experiment, as evidenced by a progressive reduction in the effects of aphid feeding on final plant biomass in each generation (Fig. 2A). In line with the expected severity of aphid feeding based on previously reported population growth rates (21), *L. erysimi* caused the strongest reduction in plant biomass, whereas *M. persicae* had an intermediate effect and *B. brassicae* had the least effect. The mixture treatment caused a reduction similar to that produced by *L. erysimi* alone, probably because aphid mixtures were dominated by this fast-growing aphid species. With each generation, trichome density decreased in the no-aphid treatment, whereas it remained at significantly higher levels in all aphid treatments (Fig. 2B). Adaptation to herbivore feeding was accompanied by considerable changes in the genotypic composition of populations, including the complete loss of nine genotypes (Fig. 3). There was a nonspecific aphid effect on total indolic glucosinolates [linear mixed effects model: $F_{1,28} = 10.66$, $P = 0.003$], with plants in the no-aphid treatment producing on average $0.98 (\pm 0.03, \text{SEM}) \mu\text{mol g}^{-1}$, and plants in aphid treatments producing $0.87 (\pm 0.03, \text{SEM}) \mu\text{mol g}^{-1}$. By contrast, the different aphid treatments had a marked effect on the dominant aliphatic chemotypes within experimental populations. Notably, the relative proportions of 3C and 4C chemotypes differed strongly among aphid treatments (Fig. 3 and fig. S4). After selection, populations of the no-aphid treatment consisted of approximately two-thirds 3C and one-third 4C chemotypes. Specialist aphids selected for different chemotypes at *GS-ELONG*: The 4C chemotypes strongly dominated in *B. brassicae* treatments [binomial generalized linear model (GLM), $t = 3.08$, $\text{df} = 25$, $P = 0.002$] and the 3C chemotypes strongly dominated in both *L. erysimi* ($t = 2.01$, $\text{df} = 25$, $P = 0.045$) and the aphid mixture treatments ($t = 2.21$, $\text{df} = 25$, $P = 0.027$). The relative proportions of 3C to 4C chemotypes in populations exposed to the generalist aphid *M. persicae* did not differ from the no-aphid treatment ($t = 0.18$, $\text{df} = 25$, $P = 0.858$). Despite this similarity, the identity of the successful genotypes differed among the two treatments, with accession Sap-0 accounting for a large fraction of plants in the no-aphid treatment but being absent from all other treatments (Fig. 3). The genotypic composition of plant populations with *L. erysimi* and aphid mixtures was near-identical, confirming that *L. erysimi* dominated the mixture treatments and suggesting that in co-founded populations, *L. erysimi* is the most important selective force. Most successful genotypes had either a 3C-OH or a 4C-NULL chemotype, and we found no individuals belonging to either alkenyl chemotype (3C-ALK or 4C-ALK) in any treatment. Alkenyl chemotypes were common in generation 1 of the selection experiment (fig. S2), and simulations of random sampling on the basis of observed population sizes revealed that their loss could not be due to drift alone (fig. S5) but rather was a consequence of selection.

To identify potential causes for the loss of particular genotypes, we measured size-standardized growth rate as a measure of fitness, together with total aliphatic glucosinolate content and trichome density in a separate experiment on all 27 ancestral accessions. Alkenyl chemotypes produced the highest amount of glucosinolates and were among the slowest growing genotypes overall (fig. S6A). Alkenyl glucosinolates are an effective defense against leaf-chewing herbivores such as caterpillars (24), but their efficiency against specialist aphids remains largely unknown, and they have little effect on *M. persicae* (23). The loss of the alkenyl chemotypes therefore probably resulted from selection against a costly defense trait that provided insufficient benefits in our experiment. This cost-benefit balance is also the most likely reason for the difference in dominant genotypes between the no-aphid treatment and the aphid treatments (Fig. 3). The dominant genotype in no-aphid populations, Sap-0, was completely absent from all aphid treatments, indicating low fitness in the presence of herbivores. The Sap-0 genotype had the lowest trichome density of all nonglabrous accessions, and as trichome production had a growth cost (fig. S6B), its success can explain the observed decrease in trichome density in the no-aphid populations over time (Fig. 2B). Compared to other 3C-OH chemotypes, Sap-0 also produces low amounts of glucosinolates, an additional indication that in the absence of herbivores, undefended, fast-growing genotypes will prosper.

Despite known epistatic interactions between *GS-ELONG* and *GS-AOP* (19), our data suggest that aphid selection acts independently on the two loci. The magnitude and direction of selection exerted by the two specialist aphids on *GS-ELONG* in our experiment suggest a causal link between the observed cline in *GS-ELONG* across Europe and the changes in the relative abundance of the same aphids. Although *B. brassicae* is numerically dominant across most of Europe, the faster-growing *L. erysimi* can inflict greater damage on plants and quickly dominates populations that are cofounded by both aphid species; thus, even a modest change in relative abundance could cause the loss of C3 populations. All plants in the selection experiment experienced strong intraspecific competition, and because growth rate is a good predictor of competitive ability (25), it is unsurprising that fast-growing plant genotypes were generally selected, whereas the slowest-growing alkenyl chemotypes were lost. Alkenyl chemotypes are, however, very common in natural populations and could be maintained by other herbivores, such as leaf-chewing caterpillars (24).

Ecological theory has consistently emphasized the role of natural enemies in maintaining diversity both within and among species, but convincing empirical evidence has been lacking. Here, we demonstrate that even functionally similar herbivores such as different species of aphid have the potential to select for specific chemotypes and

drive large-scale geographic patterns in plant defense profiles. It therefore seems likely that natural herbivore communities, with their greater variety of feeding styles and specializations, play a major role in shaping and refining the plant defenses observed in natural communities.

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Supplementary Materials

www.sciencemag.org/cgi/content/full/338/6103/116/DC1
Materials and Methods
Figs. S1 to S7
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Data Files S1 to S6

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Intestinal Inflammation Targets Cancer-Inducing Activity of the Microbiota

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Inflammation alters host physiology to promote cancer, as seen in colitis-associated colorectal cancer (CRC). Here, we identify the intestinal microbiota as a target of inflammation that affects the progression of CRC. High-throughput sequencing revealed that inflammation modifies gut microbial composition in colitis-susceptible interleukin-10-deficient (*Il10*^{−/−}) mice. Monocolonization with the commensal *Escherichia coli* NC101 promoted invasive carcinoma in azoxymethane (AOM)-treated *Il10*^{−/−} mice. Deletion of the polyketide synthase (*pks*) genotoxic island from *E. coli* NC101 decreased tumor multiplicity and invasion in AOM/*Il10*^{−/−} mice, without altering intestinal inflammation. Mucosa-associated *pks*⁺ *E. coli* were found in a significantly high percentage of inflammatory bowel disease and CRC patients. This suggests that in mice, colitis can promote tumorigenesis by altering microbial composition and inducing the expansion of microorganisms with genotoxic capabilities.

Chronic inflammation is a well-established risk factor for several cancers, including colorectal cancer (CRC) (1). Although the mechanism by which chronic intestinal inflammation leads to CRC is still unclear, numerous experimental studies suggest that inflammatory cells and their associated mediators such as interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α), IL-23, and reactive oxygen species form a microenvironment favoring the development of CRC, presumably by enhancing DNA damage in epithelial cells (2–4).

In the colon, trillions of commensal bacteria, termed “the microbiota,” are in close proximity to a single layer of epithelial cells. A critical question is whether these microorganisms actively participate in the process of carcinogenesis. We have previously shown that microbial status modulates development of colitis-associated CRC by using the colitis-susceptible *Il10*^{−/−} mouse strain (5). To evaluate the effect of inflammation and carcinogenesis on the colonic microbiota, we used Illumina (San Diego, California) HiSeq. 2000 sequencing

targeting the hypervariable V6 region of the 16S ribosomal RNA (rRNA) gene in mucosal biopsies and stool samples of *Il10*^{−/−} and wild-type (WT) mice, in the presence and absence of the colon-specific carcinogen azoxymethane (AOM). Germ-free (GF) *Il10*^{−/−} and control WT adult mice were transferred to specific pathogen-free (SPF) conditions for 20 weeks. During this time frame, 100% of *Il10*^{−/−} mice develop colitis; with the addition of AOM, 60 to 80% of mice develop colon tumors (5). WT mice developed neither colitis nor tumors (5). We first compared the luminal microbiota between all *Il10*^{−/−} (colitis/cancer) and WT mice (healthy control) and found that the microbiota of *Il10*^{−/−} mice clustered apart from those of WT controls [analysis of similarity (ANOSIM) $R = 0.925$, $P = 0.002$] (Fig. 1A and fig. S1). The altered microbiota of *Il10*^{−/−} mice showed reduced richness as compared with that of WT controls ($P < 0.0001$) (Fig. 1B). Analysis of mucosal biopsies revealed the colonic-adherent microbiota of AOM/*Il10*^{−/−} mice with colitis/cancer clustered apart from healthy AOM/WT controls (fig. S2A), with alterations in microbial evenness but not richness ($P = 0.023$) (fig. S2B). To determine the impact of AOM on the microbiota in the context of inflammation, we compared the luminal microbiota of *Il10*^{−/−} mice with colitis with that of AOM/*Il10*^{−/−} mice with colitis/cancer and found that AOM treatment had no significant effect on luminal microbial composition or richness in *Il10*^{−/−} mice (Fig. 1, C and D). These data suggest that inflammation rather than cancer is associated with the observed microbial shifts.

We then hypothesized that inflammation-induced changes in microbial composition include the expansion of bacteria within the Proteobacteria phylum because several members have been

associated with colitis and CRC (6–9). Analysis of phylum-level distribution revealed that inflammation in *Il10*^{−/−} mice was associated with significantly increased levels of luminal Verrucomicrobia, Bacteroidetes, and Proteobacteria as compared with that of WT controls (fig. S3). Although Verrucomicrobia significantly differed between groups, this phylum is not well characterized, restricting detailed molecular analysis. Within Proteobacteria, however, the Gammaproteobacteria class, Enterobacteriales order, and Enterobacteriaceae family were all significantly more abundant in *Il10*^{−/−} mice (Fig. 1, E to H). Because *E. coli* are members of the family Enterobacteriaceae and adherent-invasive *E. coli* have been associated with human inflammatory bowel disease (IBD) and CRC (8, 10–13), we determined by means of quantitative polymerase chain reaction (PCR) whether *E. coli* was more abundant in the context of inflammation in *Il10*^{−/−} mice. We found that relative to WT mice, the luminal microbiota of *Il10*^{−/−} mice exhibited a ~100-fold increase in *E. coli* (Fig. 1I). AOM treatment did not affect *E. coli* abundance (fig. S4A). Total bacterial loads between WT and *Il10*^{−/−} mice did not differ (fig. S4B), nor did levels of the common commensal *Lactobacillus* (fig. S4C).

To determine the causative effect of commensal *E. coli* on CRC, we administered AOM to GF *Il10*^{−/−} mice mono-associated with either the commensal mouse adherent-invasive *E. coli* NC101 or the human commensal *Enterococcus faecalis* OG1RF, both of which cause aggressive colitis in *Il10*^{−/−} mice (14). As expected, both *E. coli* NC101 and *E. faecalis* mono-associated, AOM-treated *Il10*^{−/−} mice developed severe colitis (Fig. 2A). Despite similar levels of colitis, 80% of *E. coli* mono-associated mice developed invasive mucinous adenocarcinoma, whereas *E. faecalis* mono-associated mice rarely developed tumors (Fig. 2, B to D). Colonic cytokines involved in inflammation and carcinogenesis including *Il6*, *Tnfa*, *Ifng*, *Il1b*, *Il18*, *Il17*, and *Il23* were not significantly different between AOM-treated *E. coli* and *E. faecalis* mono-associated mice (Fig. 2E, fig. S5, and tables S1 and S2). In addition, infiltrating CD3⁺ T cells, F4/80⁺ macrophages, and Ly6B.2⁺ monocytes and neutrophils were similar between AOM-treated *E. coli* and *E. faecalis* mono-associated mice (fig. S6). These observations demonstrate that in addition to inflammation, bacteria-specific factors may be required for the development of colitis-associated CRC.

We hypothesized that *E. coli* NC101 has carcinogenic capabilities not shared by *E. faecalis*. Several members of the family Enterobacteriaceae, including select *E. coli* strains of B2 phylotype, harbor a ~54-kb polyketide synthases (*pks*) pathogenicity island that encodes multi-enzymatic machinery for synthesizing a peptide-polyketide hybrid genotoxin named Colibactin (15–18). A bioinformatics Basic Local Alignment Search Tool (BLAST) search of the *E. coli* NC101 genome (accession NZ_AEFA00000000) revealed the presence of *pks* and the absence of other known

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E. coli genotoxins Cif, CNF, and CDT. Using PCR and sequencing (15), we detected the *pks* island in *E. coli* NC101 but not in *E. faecalis* or non-colitogenic *E. coli* K12 (Fig. 3A). To determine whether *E. coli pks* is associated with human CRC or IBD, we screened mucosa-associated *E. coli* strains isolated from colorectal tissue specimens of 35 patients with IBD, 21 with CRC, and 24 non-IBD/non-CRC controls (11). CRC specimens could not be obtained from IBD-associated CRC patients because these patients typically undergo colectomy upon diagnosis of dysplasia. Although 5 of the 24 (20.8%) non-IBD/non-CRC controls harbored *pks*⁺ *E. coli*, the genotoxic island was detected in 14 of 35 (40%, $P < 0.05$) IBD patients and in 14 of 21 (66.7%, $P < 0.001$) CRC patients (Fig. 3B and table S3). This suggests that *pks*⁺ bacteria are associated with chronic intestinal inflammation and CRC and may affect carcinogenesis.

To functionally link *pks* with the development of CRC, we created an isogenic *pks*-deficient *E. coli* NC101 strain (NC101Δ*pks*). Absence of

pks did not affect bacterial growth in vitro (fig. S7), nor did it impair colonization capacity in vivo (10^9 to 10^{10} per 200 g stool pellet, four to six mice per group). Because *pks* from strains of extraintestinal pathogenic *E. coli* can elicit mammalian DNA damage (15, 16), we tested the ability of *E. coli* NC101 *pks* to induce a DNA damage response. We infected the nontransformed rat intestinal epithelial cell line IEC-6 with NC101 or NC101Δ*pks* and assessed levels of phosphorylated histone H2AX (γH2AX), which is a surrogate marker of DNA damage (19–21). WT NC101 induced γH2AX in ~30% of cells, whereas NC101Δ*pks* induced γH2AX in <5%, which is a level equivalent to that induced by non-colitogenic *E. coli* K12 (Fig. 3C). Consistent with these results, we observed that WT NC101 induced a threefold increase in the percent of cells arrested in G₂/M phase relative to untreated and NC101Δ*pks*-infected cells (Fig. 3D). These experiments demonstrate that *pks* alone has the capacity to induce DNA damage and indicate that the genotoxic island does not block the initiation of DNA

damage response. This led us to hypothesize that *pks* would also promote tumorigenesis in vivo.

To test this hypothesis, we mono-associated GF *Il10*^{−/−} mice with *E. coli* NC101 or NC101Δ*pks*, with or without AOM treatment, and assessed inflammation and tumorigenesis. The absence of *pks* did not affect the severity of colonic inflammation in *Il10*^{−/−} mice with colitis (12 weeks with no AOM), or colitis/cancer (14 and 18 weeks with AOM) (Fig. 4A). Similarly, colon tissue pro-inflammatory cytokine transcripts and immune cell infiltration were not significantly different between mice mono-associated with NC101 versus NC101Δ*pks* (figs. S8 and S9 and table S4). However, at both 14 and 18 weeks with AOM, the absence of *pks* was associated with significantly reduced neoplastic lesions (Fig. 4B). At 14 weeks with AOM, high-grade dysplasia (HGD) or invasive carcinomas were present in five of eight mice mono-associated with NC101, whereas only one of eight NC101Δ*pks* mono-associated mice developed HGD. At 18 weeks with AOM, the absence of *pks* did not affect mouse survival or tumor size; however, macroscopic tumor burden and carcinoma invasion were significantly decreased (Fig. 4, C to F, and fig. S10). In addition, all nine NC101 mono-associated mice developed invasive carcinoma, with four of nine fully invading the muscularis propria and serosa. In contrast, zero of nine NC101Δ*pks* mono-associated mice exhibited full invasion. This likely suggests that the presence of *E. coli pks* accelerates progression from dysplasia to invasive carcinoma. In the absence of AOM, GF *Il10*^{−/−} mice colonized with NC101 for 21 weeks developed only mild dysplasia (fig. S11A). GF WT mice mono-associated with *E. coli* NC101 and treated with AOM developed neither inflammation nor dysplasia/tumors (fig. S11B), suggesting that this bacterium is not carcinogenic in the absence of inflammation. Together, these data indicate that the absence of *pks* reduces the tumorigenic potential of *E. coli* NC101 without altering colonic inflammation.

To evaluate the impact of *pks* on host DNA damage in vivo, we measured colonocyte γH2AX⁺ nuclear foci (γ-foci) in AOM/*Il10*^{−/−} mice mono-associated with NC101 versus NC101Δ*pks* for 14 weeks (19–21). The abundance of γ-foci⁺ colonocytes/crypt was significantly reduced in AOM/*Il10*^{−/−} mice mono-associated with *E. coli* NC101Δ*pks* versus *E. coli* NC101 (Fig. 4G). We detected an 80% reduction in γ-foci⁺ colonocytes/crypt in *E. coli* NC101 mono-associated AOM/WT mice relative to *E. coli* NC101-associated AOM/*Il10*^{−/−} mice (Fig. 4G). This suggests that both host inflammation and *E. coli*-derived *pks* act in concert to create a host microenvironment that promotes DNA damage and tumorigenesis in AOM/*Il10*^{−/−} mice.

Although the etiology of colitis-associated CRC is multifactorial, this work indicates that chronic inflammation targets the intestinal microbiota and can induce the expansion of microbes, including *E. coli*, that influence CRC in mice. The

Fig. 1. Inflammation alters fecal microbial community structure. (A and B) Luminal microbiota of *Il10*^{−/−} versus WT mice. (A) Operational taxonomic unit (OTU) abundances were standardized by total, square root transformed, and assembled into a Bray Curtis similarity matrix to generate a multidimensional scaling (MDS) plot, where in these plots each symbol represents the microbiota of an individual mouse analyzed by means of Illumina sequencing of 16S V6 region. *Il10*^{−/−} versus WT comparison is by ANOSIM, $R = 1$ is maximum dissimilarity. (B) Richness, mean + SEM of cage means, five to six cages per group, two to four mice per cage, t test. (C and D) Luminal microbiota of AOM/*Il10*^{−/−} versus *Il10*^{−/−} (C) MDS plot, AOM/*Il10*^{−/−} versus *Il10*^{−/−} comparison by ANOSIM. (D) Richness, mean + SEM of cage means, two to three cages per group, two to four mice per cage, t test. (E to G) Standardized transformed abundance, median + SEM of cage means, five to six cages per group, two to four mice per cage, Mann Whitney U test. (H) MDS plot depicting luminal microbiota of *Il10*^{−/−} versus WT, overlaid with *Enterobacteriaceae* abundance depicted by circle size. (I) *E. coli* Δ*ct* relative to total bacteria (16S). Each symbol depicts one mouse, line at median, Mann Whitney U test.

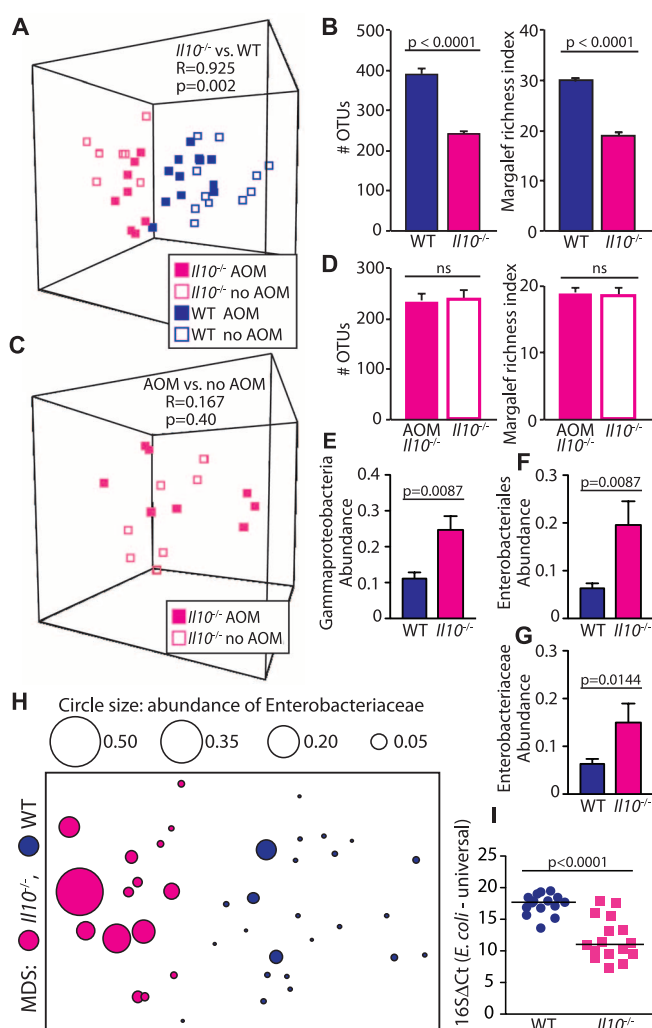


Fig. 2. Colonization of germ-free mice *Il10*^{-/-} mice with *E. coli* (*E.c.*) or *E. faecalis* (*E.f.*) differentially affects tumorigenesis without affecting inflammation. (A) Histologic inflammation scores, *t* test. (B) Macroscopic tumor counts; two-tailed Mann Whitney *U* rank sum test. (C) Percent of mice with invasive adenocarcinoma; Fisher's exact test. (D) Representative hematoxylin and eosin (H&E) histology. In (A) to (D), mean + SEM, 7 to 12 per group, single experiment. (E) Colonic cytokine mRNA expression relative to GF *Il10*^{-/-}; mean + SEM, four samples per group, *t* test.

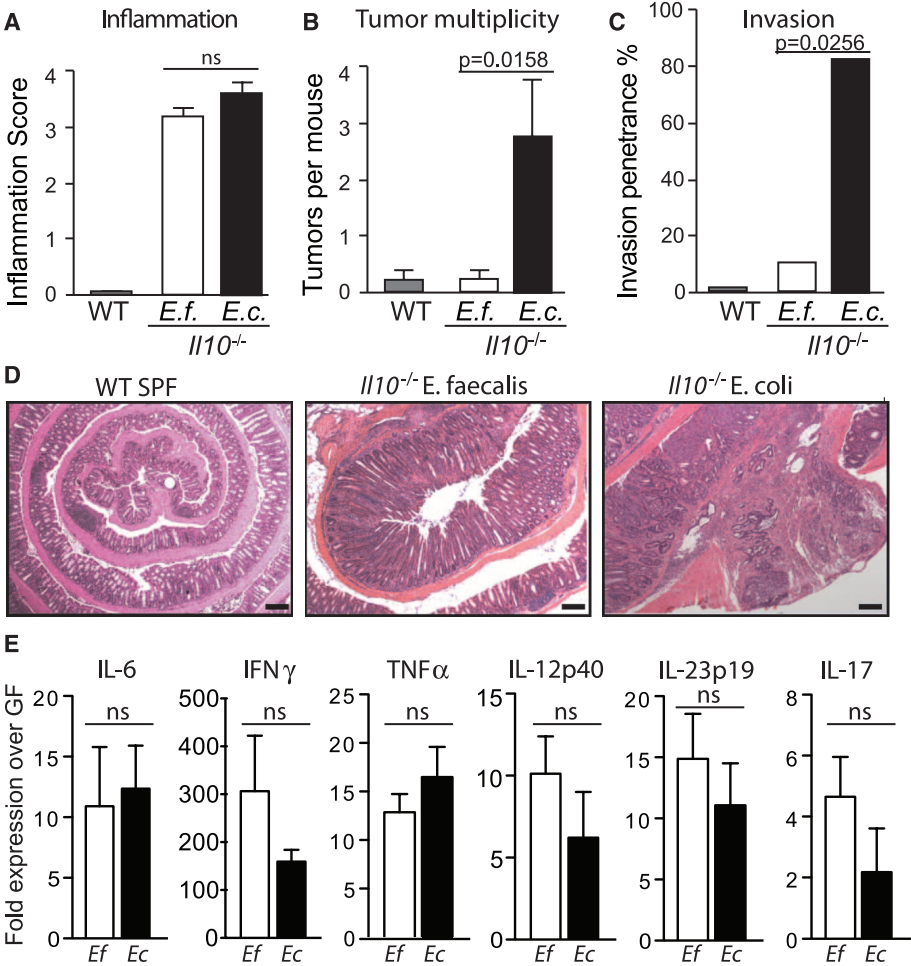
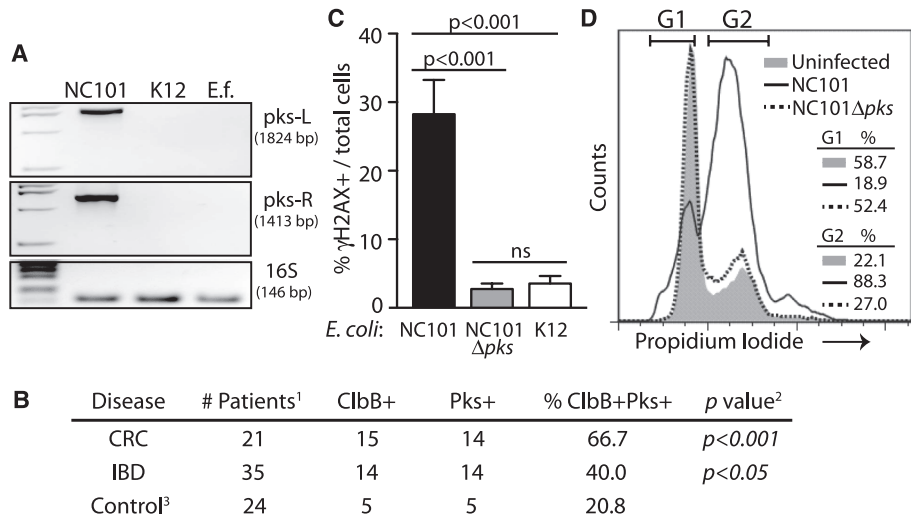


Fig. 3. *pks*⁺ *E. coli* strains are associated with CRC and DNA damage. (A and B) PCR screen for *pks* (A) using primers targeting the left [1824 base pair (bp)] and right (1413 bp) ends of the *pks* island in *E. coli* strains NC101 and K12, and *E. faecalis* (*E.f.*), and (B) using primers targeting the right end and *ClbB* gene of the *pks* island in mucosa-associated *E. coli* isolated from human colorectal tissue specimens. Binomial test **P* < 0.05, ****P* < 0.001. (C and D) NC101 *pks* induces (C) γ H2AX (MOI 20, 4 hours) in IEC-6 cells; mean + SEM, analysis of variance (ANOVA) + Tukey, and (D) G2 cell cycle arrest (MOI 100, 24 hours). (A), (C), and (D) are representative of three experiments.



carcinogenic effect of *E. coli* NC101 *pks* clearly demonstrates that genotoxic microorganisms promote CRC in the presence of the carcinogen AOM in *Il10*^{-/-} mice. It remains to be seen whether

er NC101 and other *pks*-harboring bacteria have similar effects in other models of colitis-associated CRC. An increased prevalence of *pks*⁺ *E. coli* in IBD and CRC patients may suggest a cancer-

promoting role in human CRC. We propose a model in which inflammation creates an environment that supports carcinogenesis through its effects on both the host and the microbiota. In

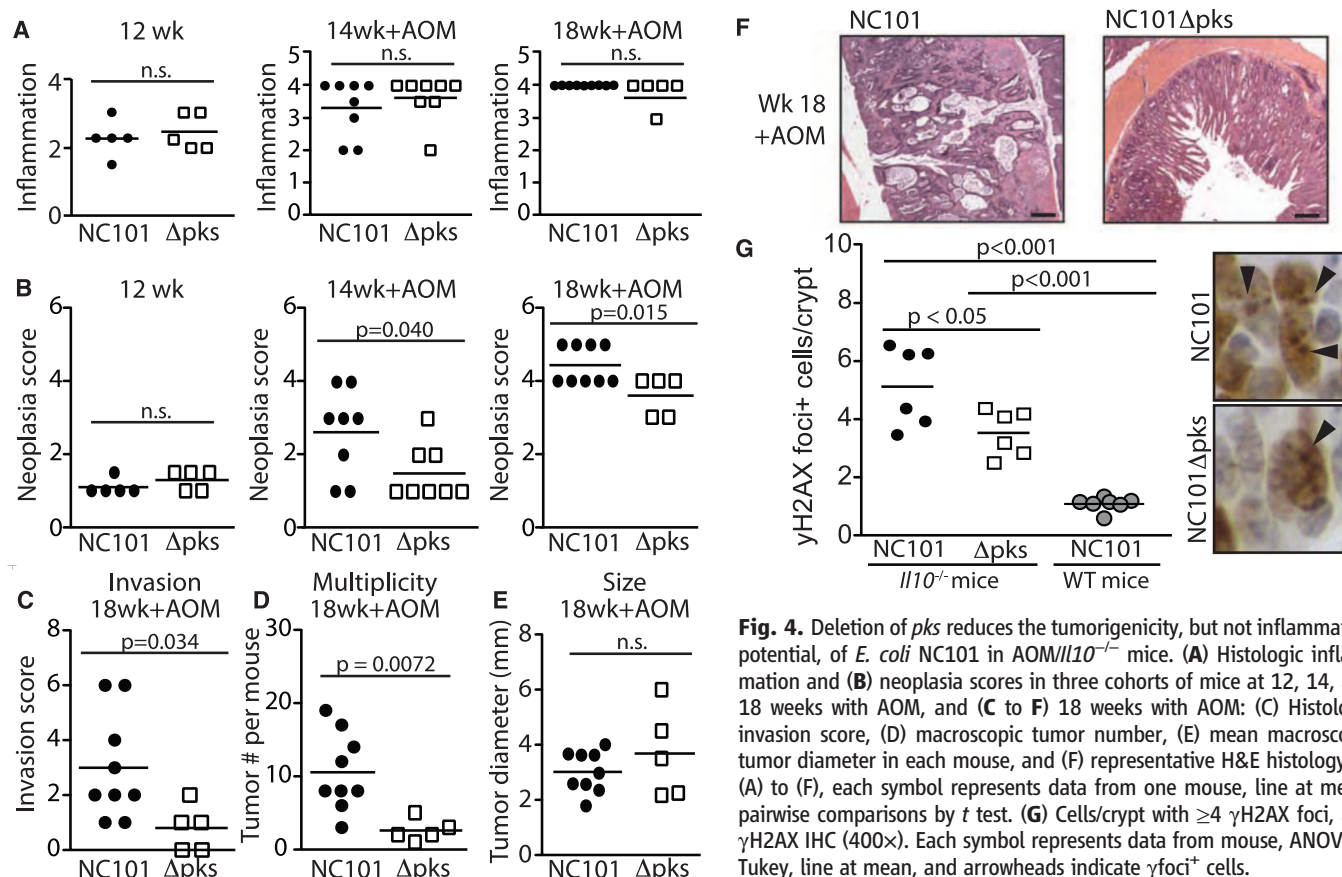


Fig. 4. Deletion of *pks* reduces the tumorigenicity, but not inflammatory potential, of *E. coli* NC101 in AOM/*Il10*^{-/-} mice. (A) Histologic inflammation and (B) neoplasia scores in three cohorts of mice at 12, 14, and 18 weeks with AOM, and (C to F) 18 weeks with AOM: (C) Histologic invasion score, (D) macroscopic tumor number, (E) mean macroscopic tumor diameter in each mouse, and (F) representative H&E histology. In (A) to (F), each symbol represents data from one mouse, line at mean, pairwise comparisons by *t* test. (G) Cells/crypt with ≥ 4 γ H2AX foci, and γ H2AX IHC (400 $\times$). Each symbol represents data from mouse, ANOVA + Tukey, line at mean, and arrowheads indicate γ foci⁺ cells.

this two-hit model, inflammation targets the microbiota to foster the expansion of bacteria with genotoxic potential, such as *pks*⁺ bacteria. In parallel, inflammation creates an opportunity for *pks*⁺ bacteria to adhere to the colonic mucosa by decreasing protective mucins and antimicrobial peptide production (22–24)—a process prevented by natural barrier function present in noninflamed WT mice. The genotoxic effect of *pks* requires bacteria-host cell contact (15, 16); thus, an environment in which bacteria can more readily access the epithelium could result in increased delivery of the *pks* product Colibactin to epithelial host cells. This would explain the lack of cancer in *pks*⁺ *E. coli*-associated WT mice. Although other microbes likely participate in the progression of CRC, our findings highlight the complex effects of inflammation on both microbial composition/activity and the host's ability to protect itself from a dysbiotic microbiota (25).

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25. Materials and methods are available as supplementary materials on Science Online.

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DK034987). This work was supported by funding from NIH T32 DK007737 (J.C.A.), R01 DK73338 (C.J.), R01 DK47700 (C.J.), R01 CA136887 (T.O.K.), R01 DK53347-11 (K.W.S./A.B.S.), the American Institute for Cancer Research (C.J.), UNC University Cancer Research Fund (C.J.), New York Presbyterian/Weill Cornell Medical College (K.W.S.), Crohn's and Colitis foundation UK (B.J.C.), the NIH Research Specialist Biomedical Research Center in Microbial Disease (J.M.R.), the North West Cancer Research Fund UK (B.J.C.), and the Canadian Institutes of Health Research MOP#114872 (A.S.). T.A. is supported by a scholarship from King Abdulaziz University, through the Saudi Arabian Cultural Bureau in Canada. J.M.R. is a member of advisory boards for Atlantic, Procter and Gamble, and Falk and has received speaking honoraria from Abbott, Falk, Ferring, GlaxoSmithKline, Procter and Gamble, Schering Plough, Shire, and Wyeth. All data presented in this manuscript are tabulated in the main paper and in the supplementary materials. Illumina sequencing data are deposited in National Center for Biotechnology Information's (NCBI's) sequence read archive (SRA) and are accessible under SRA055272. Inflammation PCR array data are deposited in NCBI's Gene Expression Omnibus (GEO) and are accessible through GEO series accession no. GSE39085. This work is in the memory of Mathieu Jobin, Lyne Gauthier, and Janine Drysdale.

Supplementary Materials

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Materials and Methods
Figs. S1 to S11
Tables S1 to S4
References (26–39)

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BDNF Is a Negative Modulator of Morphine Action

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Brain-derived neurotrophic factor (BDNF) is a key positive regulator of neural plasticity, promoting, for example, the actions of stimulant drugs of abuse such as cocaine. We discovered a surprising opposite role for BDNF in countering responses to chronic morphine exposure. The suppression of BDNF in the ventral tegmental area (VTA) enhanced the ability of morphine to increase dopamine (DA) neuron excitability and promote reward. In contrast, optical stimulation of VTA DA terminals in nucleus accumbens (NAc) completely reversed the suppressive effect of BDNF on morphine reward. Furthermore, we identified numerous genes in the NAc, a major target region of VTA DA neurons, whose regulation by BDNF in the context of chronic morphine exposure mediated this counteractive function. These findings provide insight into the molecular basis of morphine-induced neuroadaptations in the brain's reward circuitry.

Brain-derived neurotrophic factor (BDNF) is a positive modulator of many forms of neural plasticity throughout the adult nervous system (1, 2). In the context of drug addiction, BDNF is best characterized for its role in promoting the neural and behavioral plasticity induced by cocaine or other stimulants via actions on the mesolimbic dopamine (DA) system, a key reward circuit in the brain, where the BDNF pathway is engaged in a feed-forward loop that promotes further actions of stimulant drugs (3–8).

Opiates also act on the ventral tegmental area (VTA) and nucleus accumbens (NAc) to produce reward acutely and addiction chronically. However, there are differences in the cellular actions of opiates versus stimulants on this reward circuit. Stimulants promote DA signaling in the NAc primarily by acting on DA terminals in this region to increase extracellular DA levels. In contrast, opiates promote DA signaling to the NAc by inhibiting local γ -aminobutyric acid (GABA) interneurons in the VTA, which then disinhibit (activate) VTA DA neurons (9); opiates also act via DA-independent mechanisms (10). Chronic opiates induce some unique biochemical and morphological alterations in the VTA. Although the effect of opiates on BDNF expression in the VTA is inconsistent (10–12), opiates down-regulate intracellular BDNF signaling cascades and reduce the soma size of VTA DA

neurons (13–16), effects not seen with stimulants. Some of these biochemical and morphological changes in the VTA are reversed by direct administration of BDNF into this brain region (13, 16). This suggests that, opposite to the situation for cocaine and other stimulants, opiate and BDNF actions might converge by producing counteractive effects on VTA DA neurons. This led us to hypothesize an antagonistic role for endogenous BDNF-TrkB signaling in modulating adaptive responses of the VTA-NAc pathway to chronic opiate exposure.

We first demonstrated that chronic morphine, whether given to mice by subcutaneous pellets or intermittent intraperitoneal injections, decreased BDNF expression in the VTA (fig. S1). Next, we examined the role of BDNF-TrkB signaling in the VTA-NAc in morphine action by performing morphine-conditioned place preference (CPP), which provides an indirect measure of drug reward. Morphine doses were selected on the basis of an initial dose-response analysis (fig. S2). Infusion of an adeno-associated virus (AAV) vector encoding Cre recombinase fused to green fluorescent protein (GFP) (AAV-CreGFP) into the VTA of mice homozygous for *bdnf* or *trkB* genes flanked by loxP sites [floxed BDNF (fBDNF) or floxed TrkB (fTrkB) mice] produced highly localized CreGFP expression in this brain region (fig. S3A). This induced a 40 to 60% reduction of BDNF or TrkB mRNA levels in the VTA, as compared with control animals injected with AAV-GFP (fig. S3, B and C). Localized knockdown of BDNF enhanced the rewarding effect of morphine at both subthreshold doses [5 mg per kilogram of body weight (mg/kg); fig. S3D] and higher doses (15 mg/kg; Fig. 1A) as compared with AAV-GFP controls. Equivalent effects were seen for local VTA knockdown of TrkB (Fig. 1B, 15 mg/kg). There were no differences in baseline preference scores before conditioning among the fBDNF and fTrkB groups.

Because the viral-mediated knockdown procedure affects both DA and non-DA VTA neurons, we used a complementary approach to knock out

TrkB selectively from DA neurons by crossing fTrkB mice with DA transporter (DAT)-Cre mice (*TrkB^{lox/lox};DAT^{cre/wt}*) (17). Selective ablation of TrkB from DA neurons in *TrkB^{lox/lox};DAT^{cre/wt}* mice increased morphine reward (Fig. 1C). There were no differences in baseline or morphine CPP among several control groups examined, which included wild-type mice (*TrkB^{wt/wt};DAT^{wt/wt}*), floxed control mice (*TrkB^{lox/lox};DAT^{wt/wt}*), and Cre control mice (*TrkB^{wt/wt};DAT^{cre/wt}*), indicating that the increase in morphine reward seen upon selective TrkB ablation in DA neurons does not result from allele-specific effects. In contrast, a single intra-VTA infusion of BDNF (0.25 μ g per side) decreased morphine CPP as compared with vehicle-infused control animals (Fig. 1D).

BDNF synthesized in VTA DA neurons can undergo anterograde transport and release in the NAc to activate TrkB receptors on NAc neurons (18). We therefore tested the effect on morphine reward of localized deletion of TrkB receptors in the NAc of fTrkB mice and of intra-NAc infusions of BDNF (1.0 μ g per side). In contrast to the VTA, TrkB knockdown (Fig. 1E) and BDNF infusion (Fig. 1F) in the NAc had no effect on morphine CPP. It is thus local BDNF signaling in VTA DA neurons that is responsible for the regulation of morphine reward.

We have recently shown that chronic morphine decreases the expression of certain K^+ channels in the VTA, such as *kcnab2*, *kcnj2*, and *girk3*, and that such adaptations are associated with increased excitability of DA neurons (14). Viral-mediated BDNF knockdown in the VTA similarly suppressed mRNA levels of these and some additional K^+ channels (fig. S4A). These findings raise the possibility that morphine increases VTA DA neuron excitability via down-regulation of BDNF and the subsequent reduction in K^+ channel expression. To test this hypothesis, we obtained extracellular single-unit recordings from VTA DA neurons in three groups of anesthetized mice: controls, chronic morphine, and chronic morphine+intra-VTA BDNF-infused (Fig. 2A). Consistent with previous ex vivo findings from brain slices (14), morphine increased the in vivo spontaneous firing rates of VTA DA neurons by 44%. Intra-VTA infusion of BDNF normalized this morphine-induced firing rate increase (Fig. 2B). Analysis of burst phasic firing, which substantially increases DA release as compared to single-spike tonic firings (19, 20), revealed that overall bursting events were increased by chronic morphine and restored by intra-VTA BDNF (Fig. 2, C and D; fig. S4, B and C). Conversely, localized VTA BDNF knockdown alone (in morphine-naïve mice) increased the spontaneous burst firing of DA neurons (Fig. 2, G to I).

Next, we studied possible ionic mechanisms underlying these changes, using standard whole-cell voltage-clamp recordings. Different components (peak and sustained) of K^+ currents in VTA DA neurons were reduced by morphine treatment, which were blocked by intra-VTA BDNF (Fig. 2, E and F). These data demonstrate that

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morphine and BDNF differentially regulate K^+ currents in these neurons, which is consistent with our molecular findings above. The ability of chronic morphine to excite VTA DA neurons may be mediated via decreased AKT signaling (14), which would be an expected downstream consequence of the withdrawal of BDNF support. Prior work suggested as well that down-regulation of BDNF signaling in the VTA would excite VTA DA neurons further by reducing GABA_A receptor responses, also downstream of reduced AKT signaling (21). Our findings dem-

onstrate that BDNF additionally controls the intrinsic excitability of VTA DA neurons via altered K^+ channel expression, and thereby opposes morphine-induced DA neuron excitability through a homeostatic scaling mechanism (22, 23).

Given these direct links between VTA BDNF expression and VTA DA neuron excitability in morphine action, we next determined whether BDNF-regulated activity of VTA DA neurons is important for BDNF's influence on behavioral responses to morphine. We stereotactically delivered AAV-ChR2 (channel rhodopsin)-EYFP

(enhanced yellow fluorescent protein) or AAV-EYFP into the mouse VTA as described (24). Two to 3 weeks later, when AAV expression was maximal, we infused BDNF into the VTA and implanted cannulae in the NAc for optical fiber placement (Fig. 3A and fig. S5A). Animals were studied 1 week later. 86% of ChR2-EYFP-positive cells in the VTA colocalized with TH, a marker of DA neurons (fig. S5B). ChR2-EYFP immunoreactivity in the NAc colocalized with DAT, a marker of DA nerve terminals (Fig. 3, B to D), but not with GAD67 (Fig. 3, E to G) or VGLUT2 (Fig. 3, H to J), markers of GABA or glutamate terminals, respectively, showing selective expression of ChR2-EYFP in DA nerve terminals in the NAc. Mice that expressed AAV-ChR2-EYFP or AAV-EYFP in the VTA were studied in the morphine CPP model by conditioning them with subthreshold morphine doses (10 mg/kg) plus 20-Hz phasic pulses delivered to the NAc in one chamber, and with saline and no light in the opposite chamber. In non-BDNF-infused animals, this optogenetic protocol induced significant morphine CPP in mice expressing ChR2-EYFP but not EYFP alone (Fig. 3K). Such stimulation also completely prevented the inhibitory effect of intra-VTA BDNF infusion on morphine CPP (Fig. 3K, compare to Fig. 1D and fig. S6A). In this model, light stimulation of DA terminals in NAc, at 20 Hz or several other frequencies, in the absence of morphine did not induce a CPP regardless of intra-VTA BDNF infusion (fig. S6, C to E). The effect of light stimulation was mediated by D1 DA receptors in the NAc, because intra-NAc injection of a D1 receptor antagonist (SCH 23390, 1 μ g), at a dose known to be behaviorally active (25, 26), completely blocked the ability of light stimulation to enhance morphine CPP regardless of intra-VTA BDNF infusion (Fig. 3L). In contrast, intra-NAc injection of behaviorally active doses (27–29) of a D2 (eticlopride, 1 or 4 μ g) or glutamate [6,7-nitroquinoxaline-2,3-dione (DNQX), 1 or 4 μ g; or 6-nitro-7-sulfamoylbenzo[*f*]quinoxaline-2,3-dione (NBQX), 400 ng] receptor antagonist had no effect (fig. S7). These data demonstrate that BDNF impairment of morphine reward can be rescued by phasic stimulation of the VTA-NAc DA pathway, specifically through D1 receptors in the NAc. This is consistent with evidence showing that DA released by burst-like phasic firing selectively binds to D1 receptors (19, 30). In contrast to the NAc, optical stimulation of DA terminals in the medial prefrontal cortex had no effect on morphine CPP (fig. S8).

We investigated the downstream consequences of VTA BDNF and chronic morphine on gene expression in the NAc. We performed microarray analysis on the NAc from mice in which VTA BDNF was virally knocked down, with half of the animals then treated chronically with morphine. We identified clusters of NAc genes that are regulated by morphine or by knock-down of VTA BDNF and analyzed interactive effects between the two to investigate the molecular

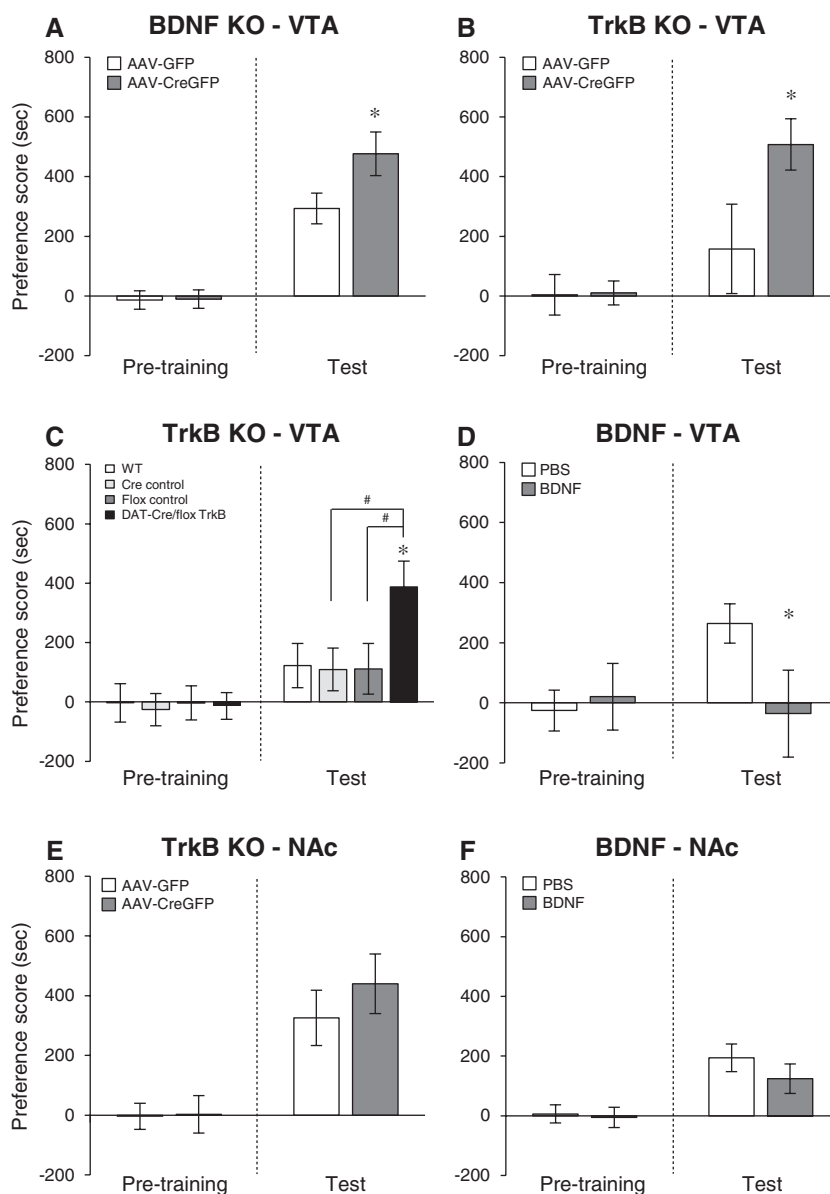
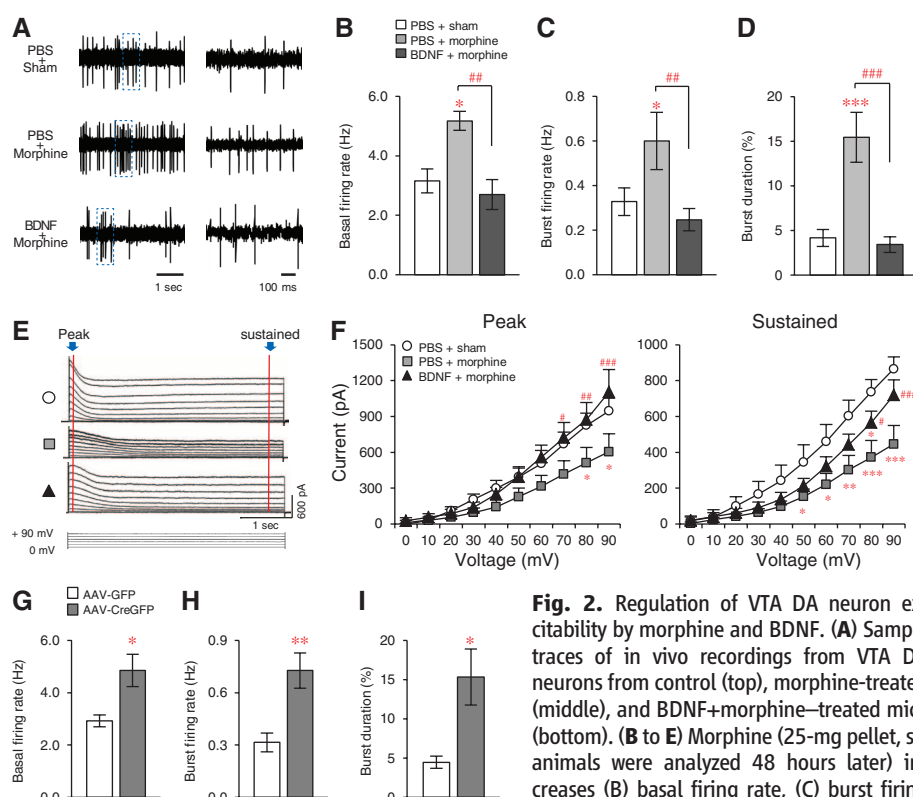
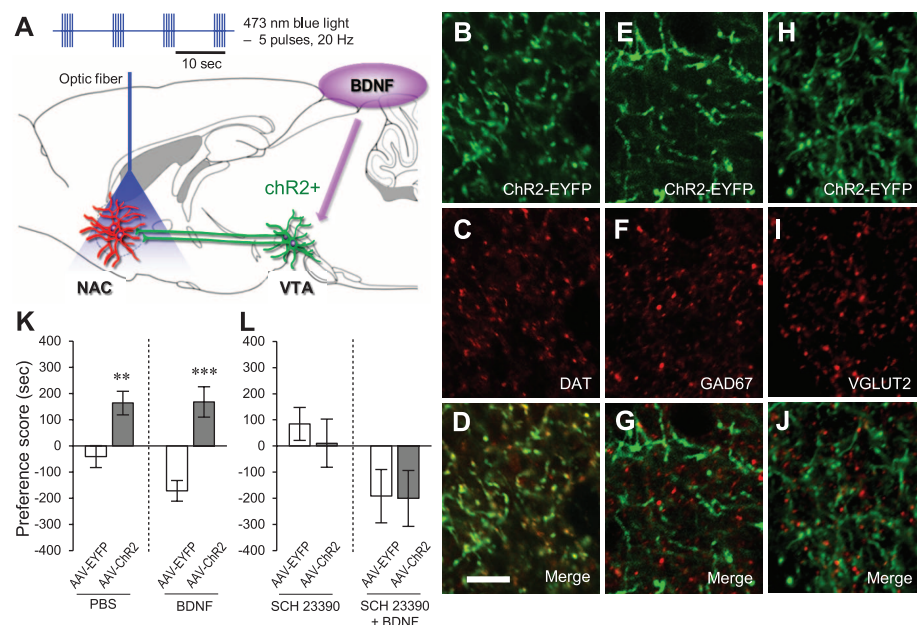


Fig. 1. Effects of BDNF-TrkB signaling within the VTA-NAc on morphine reward. Localized knockout (KO) of BDNF (A) or TrkB (B) from VTA neurons enhances morphine CPP [15 mg/kg, subcutaneous (sc)]. Student's *t* test, $*P < 0.05$, $n = 8$ to 12 mice. (C) DAT-Cre/flox TrkB ($TrkB^{lox/lox}; DAT^{cre/wt}$) mice also displayed enhanced morphine CPP [10 mg/kg, intraperitoneal (ip)]. One-way analysis of variance (ANOVA), Fisher's protected least significant difference (PLSD) post-hoc test, $*P < 0.05$ compared to controls; $\#P < 0.05$ compared with $TrkB^{lox/lox}; DAT^{cre/wt}$ mice, $n = 6$ to 11 mice. (D) A single infusion of BDNF into the VTA (0.25 μ g per side) suppressed morphine CPP (15 mg/kg, sc). PBS, phosphate-buffered saline. *t* test, $*P < 0.05$, $n = 7$ or 8 mice. (E) Localized TrkB KO in the NAc and (F) intra-NAc BDNF infusion (1.0 μ g per side) had no effect on morphine CPP (15 mg/kg, sc), $n = 8$ or 9 mice.



reons, which were normalized by intra-VTA infusion of BDNF (0.25 μ g per side). One-way ANOVA, Fisher's PLSD test, $^*P < 0.05$, $^{***}P < 0.001$ compared with control; $^{##}P < 0.01$, $^{###}P < 0.001$ compared with the morphine group. $n = 4$ to 6 mice. (E) Sample traces of K^+ conductance recorded from VTA DA neurons in brain slices from control, morphine-treated, and BDNF+morphine-treated mice. (F) Morphine treatment as in (A) significantly decreased both peak and sustained phases of K^+ currents in VTA DA neurons, an effect that was reversed by BDNF. Two-way ANOVA, Fisher's PLSD test, $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ compared with the morphine group. $n = 5$ to 9 mice. Localized BDNF KO from VTA increases (G) basal firing rate, (H) burst firing rate, and (I) burst duration in VTA DA neurons. t test, $^*P < 0.05$, $^{**}P < 0.01$ compared with AAV-GFP controls, $n = 7$ mice.

Fig. 3. Modulation of morphine reward by optogenetic activation of DA terminals in the NAC. (A) Experimental model for optogenetic stimulation during morphine CPP. Mice were conditioned to a morphine/light chamber and a saline/no-light chamber for 30 min. 470-nm phasic light pulses (20 Hz, five pulses, 40 ms duration) were delivered during the 30-min conditioning session. (B to J) Immunostaining for ChR2-EYFP [(B), (E), and (H), green], DAT [(C), red], GAD67 [(F), red], and VGLUT2 [(I), red] in the NAC. (D), (G), and (J) Confocal microscopy shows that ChR2-EYFP puncta in NAC co-label for DAT, but not GAD67 or VGLUT2. Scale bar, 10 μ m. (K) In vivo optogenetic stimulation of VTA DA nerve terminals in the NAC enhances morphine reward (10 mg/kg, ip) and prevents VTA BDNF-induced impairment of morphine reward, (L) whereas D1 receptor antagonism (SCH 23390, 1 μ g) blocks light potentiation, and VTA BDNF-induced impairment, of morphine reward. t test, $^{**}P < 0.01$, $^{***}P < 0.001$, $n = 7$ to 11 mice.



mechanisms underlying BDNF regulation of morphine responses. Three main categories of genes were identified (table S1): category A, genes regulated by morphine, in which that regulation is lost upon knockdown of VTA BDNF (Fig. 4A); category B, genes whose regulation by morphine was uncovered upon knockdown of VTA BDNF (Fig. 4A); and category C, genes regulated by morphine regardless of VTA BDNF knockdown (Fig. 4B) (see table S2 for complete gene lists). Among the genes significantly regulated in the NAc under these conditions are several that have previously been implicated in morphine action, such as *zfp40*, *xdh*, *nt5e*, *sult1a1*, *gadd45g*, *rbm3*, and *zbtb16* (31–34). We also found a significantly greater transcriptional effect of chronic morphine in the NAc of mice lacking BDNF in the VTA: About three times more genes (151 versus 46) were regulated by morphine in VTA BDNF knockdown mice than in control mice (fig. S9, A to C). Similarly, about five times more genes (185 versus 39) in the NAc were regulated upon knockdown of BDNF in the VTA in morphine-treated mice than in sham mice (fig. S9, D to F) (see table S3 for complete gene lists). These findings extend our behavioral and electrophysiological evidence that BDNF in the VTA antagonizes chronic morphine actions on the VTA-NAc circuit.

We selected two genes, *sox11* and *gadd45g*, from categories A and B, respectively, for further study and validated their expression patterns using reverse transcription polymerase chain reaction (RT-PCR) analysis (Fig. 4, D to G). SOX11 is a transcription factor involved in embryonic neurogenesis and tissue remodeling (35). The function of the gene in the adult nervous system remains unknown, although its regulation was observed in a previous microarray study on the NAc (36). We found that *sox11* gene expression levels in the NAc were induced by chronic morphine and that

this increase was prevented by the deletion of BDNF in the VTA (Fig. 4, D and E).

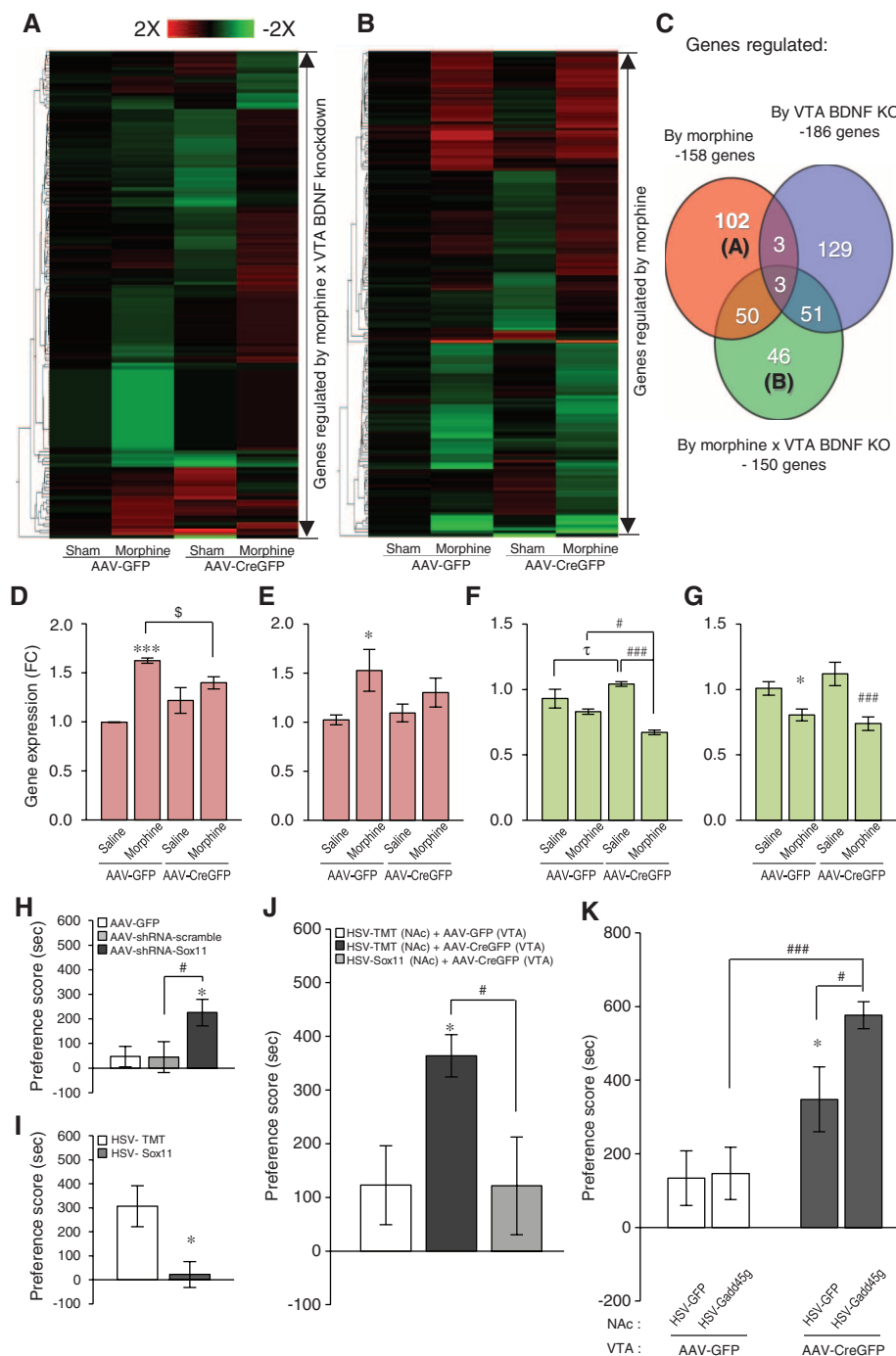
To directly test whether alterations in *sox11* expression in the NAc influence morphine reward, we generated an AAV vector that expresses a short-hairpin RNA (shRNA) against *sox11* to knock it down in the NAc selectively and a herpes simplex virus (HSV) vector to overexpress *sox11* in this region (fig. S10A). After first validating the AAV-shRNA-*Sox11* vector in cultured Neuro2A cells (fig. S10, B and C), we demonstrated that intra-NAc infusion of this vector reduced *sox11* expression in the NAc (fig. S10D). In contrast, intra-NAc infusion of HSV-*Sox11*

robustly augmented *sox11* mRNA levels in this region (fig. S10E). We observed that AAV-shRNA-*Sox11* in the NAc increased morphine reward: A subthreshold dose of morphine (10 mg/kg) induced significant CPP, an effect not seen in animals treated with control AAV-GFP or AAV-scrambled shRNA vectors (Fig. 4H). Conversely, HSV-*Sox11* in the NAc decreased CPP to a higher dose of morphine (15 mg/kg) as compared to HSV-tomato-infused control animals (Fig. 4I). Furthermore, the ability of locally knocking down BDNF-TrkB signaling in the VTA to enhance morphine's rewarding effects was completely normalized upon HSV-mediated *sox11* overexpression in the NAc (Fig.

4J). No changes were observed in baseline levels of place preference in these experiments.

Another gene implicated in these interactions by our microarray data is *gadd45g*, a stress-responsive immediate early gene, which is involved in DNA repair, cell growth arrest, and apoptosis (37). Here, *gadd45g* gene expression levels were more robustly and consistently suppressed in the NAc by chronic morphine in mice with deletion of VTA BDNF (Fig. 4, F and G). We then tested whether such regulation of *gadd45g* expression in the NAc influences the rewarding effects of morphine using HSV-*Gadd45g* that we developed and validated for *gadd45g* overexpression (fig. S11).

Fig. 4. Morphine-regulated NAc gene expression after VTA BDNF deletion: Identification of NAc mediators of BDNF-morphine interactions. Microarray analysis was performed on the NAc of sham- and morphine-pelleted mice under control or VTA BDNF knockdown conditions. (A) Heat map of up-regulated (red) or down-regulated (green) NAc genes upon knockdown of VTA BDNF. (B) Heat map of up- or down-regulated NAc genes by morphine regardless of knockdown of VTA BDNF. (C) Venn diagrams of genes that were regulated by morphine (red) or by knockdown of VTA BDNF (blue), and of genes that were regulated by morphine and knockdown of VTA BDNF in an interactive manner (green). (D to G) Alterations of *sox11* [(D) and (E)] and *gadd45g* [(F) and (G)] expression in the NAc from a heat map of microarray analysis [(D) and (F)] and RT-PCR (RT-PCR) validation [(E) and (G)]. One-way ANOVA for RT-PCR validation, Fisher's PLSD test, $^*P < 0.1$, $^{**}P < 0.05$, $^{***}P < 0.001$ compared to sham+AAV-GFP controls; $^{\$}P < 0.1$, $^{\#}P < 0.05$, $^{\#\#}P < 0.001$ compared to sham+AAV-CreGFP mice, $n = 9$ to 12 mice. (H) Reduction of *sox11* expression using AAV-shRNA-*Sox11* increases morphine CPP (10 mg/kg, sc). Fisher's PLSD test, $^*P < 0.05$ compared with AAV-GFP controls; $^{\#}P < 0.05$ compared with AAV-scrambled controls, $n = 11$ or 12 mice. (I) *Sox11* overexpression in NAc using HSV-*Sox11* decreases morphine CPP (15 mg/kg, sc). t test, $^*P < 0.05$, $n = 8$ mice. (J) Enhancement of morphine reward (15 mg/kg, sc) induced by knockdown of VTA TrkB is counteracted by *sox11* overexpression in the NAc. Fisher's PLSD test, $^*P < 0.05$ compared with HSV-TMT (NAc)+AAV-GFP (VTA) controls; $^{\#}P < 0.05$ compared with HSV-TMT (NAc)+AAV-CreGFP (VTA) mice. (K) Enhancement of morphine reward (12.5 mg/kg, sc) induced by knockdown of VTA TrkB is further enhanced by *gadd45g* overexpression in the NAc. Fisher's PLSD test, $^*P < 0.05$ compared to HSV-GFP+AAV-GFP controls; $^{\#}P < 0.05$, $^{\#\#}P < 0.001$ compared to HSV-*Gadd45g*+AAV-CreGFP.



HSV-Gadd45g infusion into the NAc of normal mice did not affect the rewarding actions of a moderate dose of morphine (12.5 mg/kg). However, *gadd45g* overexpression in the NAc of mice with local VTA knockdown of TrkB enhanced morphine CPP as compared to HSV-GFP treated mice (Fig. 4K). No changes were observed in baseline levels of place preference.

We observed the opposite effect of BDNF-TrkB signaling in the VTA-NAc on morphine reward to that seen in earlier observations with cocaine and other stimulants. Prior work has shown that knockdown of BDNF from the VTA, or knockdown of BDNF or TrkB from the NAc, antagonizes behavioral responses to cocaine, whereas knockdown of TrkB in the VTA has no effect (3). These findings identified the NAc as the primary site of action of BDNF-TrkB signaling in regulating cocaine reward. In contrast, we show here that the primary site of action of BDNF-TrkB signaling in regulating morphine reward is the VTA, because knockdown of either BDNF or TrkB in the VTA promotes behavioral responses to morphine, whereas knockdown of TrkB in the NAc and BDNF administration into the NAc were without effect. On the other hand, our findings that optical stimulation of VTA DA nerve terminals in the NAc completely reverses the ability of BDNF, acting in the VTA, to impair morphine reward demonstrate that BDNF's influence on VTA DA neuron excitability is responsible for its behavioral effects reported here (fig. S12). These optogenetic experiments also identify the NAc as the key neural site where VTA BDNF's influence on morphine reward is ultimately mediated, and we showed that this occurs via DA activation of D1 receptors on NAc neurons. For this reason, we analyzed morphine-induced changes in gene expression in the NAc as a function of VTA BDNF and demonstrated the importance of two genes, *sox11* and *gadd45g*, among numerous other putative genes regulated in a similar fashion, in behavioral responses to morphine (fig. S12). The results of the present study differ from those in an earlier report (10), which found that exogenous BDNF increases opiate reward in rats by altering GABA_A receptor function in VTA GABAergic neurons (fig. S12). The different findings could be due to the different species, drug treatment regimens, or behavioral models used.

Consistent with the differences in BDNF's influence on cocaine versus morphine action are the very different ways in which the drugs initially affect the VTA-NAc pathway. In addition, although opiate and stimulant drugs of abuse induce many common molecular and cellular adaptations in both the VTA and NAc (38), some notable differences are seen as well with respect to synaptic plasticity (39, 40) and to VTA BDNF expression (fig. S1B). Further studies are now needed to directly investigate whether the opposite interactions between BDNF and stimulants versus opiates are related to these adaptations. Given the substance-specific features of drug addiction syndromes, it will be important to further explore the downstream functional consequences of the

BDNF-stimulant feed-forward loop versus the BDNF-opiate negative feedback loop in addiction and to study how they influence polydrug use.

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Supplementary Materials

www.sciencemag.org/cgi/content/full/338/6103/124/DC1
Materials and Methods
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Shared Synaptic Pathophysiology in Syndromic and Nonsyndromic Rodent Models of Autism

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The genetic heterogeneity of autism poses a major challenge for identifying mechanism-based treatments. A number of rare mutations are associated with autism, and it is unclear whether these result in common neuronal alterations. Monogenic syndromes, such as fragile X, include autism as one of their multifaceted symptoms and have revealed specific defects in synaptic plasticity. We discovered an unexpected convergence of synaptic pathophysiology in a nonsyndromic form of autism with those in fragile X syndrome. Neuroligin-3 knockout mice (a model for nonsyndromic autism) exhibited disrupted heterosynaptic competition and perturbed metabotropic glutamate receptor-dependent synaptic plasticity, a hallmark of fragile X. These phenotypes could be rescued by reexpression of neuroligin-3 in juvenile mice, highlighting the possibility of reverting neuronal circuit alterations in autism after the completion of development.

Autism comprises a heterogeneous group of neurodevelopmental disorders characterized by variations in social interactions and communication and the manifestation of ritualistic behaviors (1). A large number of rare high-impact mutations have been identified in autistic patients (2–4). However, most insights into the synaptic pathophysiology of autism are derived from models of monogenic syndromes, such as fragile X syndrome, in which about 25%

of patients meet diagnostic criteria of autism (5). In fragile X, the key defect in synaptic transmission is elevated group I metabotropic glutamate receptor-dependent long-term depression (mGluR-LTD). However, most cases of autism are nonsyndromic, and it is unclear whether these share pathophysiology with fragile X. One class of nonsyndromic forms of autism is associated with mutations in the neuroligin genes (*Nlgn1*, -2, -3, and -4), which encode postsynaptic adhesion molecules

involved in synapse assembly (proteins NL1, -2, -3, and -4) (6–8). For *Nlgn3*, a R451C point mutation (7) and deletions (4, 9) have been identified in several patients with autism. The R451C point mutation results in NL3 trafficking defects (10), whereas the deletions remove the entire *Nlgn3* coding sequence (4, 9). *Nlgn3*^{R451C} knockin and *Nlgn3*^{KO} (KO, knockout) mice exhibit impairments in social interactions, social memory, ultrasonic vocalization, and olfaction [(11, 12) but see (13)]. In *Nlgn3*^{R451C} mice, synaptic transmission is altered in the somatosensory cortex and hippocampus (12, 14). However, the subcellular localization of NL3 protein in vivo and synaptic defects resulting from *Nlgn3* ablation are unclear (15, 16). To understand the pathophysiology of *Nlgn3* deletions, we focused on the synaptic connectivity in the cerebellum, because cerebellar activation is altered in autistic patients (17), and

cerebellar lesions result in behavioral changes reminiscent of autism (18, 19). Using NL3-specific antibodies (16), we detected strong NL3 immune reactivity in the molecular layer and surrounding mossy fiber glomeruli of the inner granular layer (Fig. 1, A and B). Antibody reactivity was largely abolished in mutant mice carrying a STOP cassette inserted after the transcriptional start site (20), which resulted in a complete loss of NL3 expression (*Nlgn3*^{KO}) (Fig. 1A and fig. S1, A and B). In the inner granular layer, NL3 was detected at glutamatergic as well as γ -aminobutyric acid-responsive (GABAergic) synapses, whereas NL3 in the molecular layer was primarily detected at parallel fiber synapses (Fig. 1, C and D). In the same preparations, we detected only little apposition of NL3 immune reactivity with markers of climbing fiber and interneuron synapses (fig. S1C). Similar observations were made in *Nlgn3*^{PC} mice, carrying a knockout allele where NL3 is selectively reexpressed in Purkinje cells (PCs) under the control of the tetracycline transactivator (Fig. 1, A and B, and fig. S1D).

Ultrastructural analysis of parallel fiber synapses in *Nlgn3*^{KO} mice did not reveal a dramatic difference in several morphological parameters (Fig. 2A). Miniature excitatory postsynaptic current (mEPSC) recordings in *Nlgn3*^{KO} PCs identified a small but significant reduction in mEPSC

amplitude (Fig. 2B), whereas paired-pulse facilitation of parallel fiber synapses was not detectably altered (Fig. 2C and fig. S2A). Parallel fiber synapses exhibit a marked LTD that is thought to contribute to forms of cerebellar function and learning [(21) but see (22)]. Simultaneous activation of group I mGluRs and AMPA receptors in the postsynaptic compartment results in protein kinase C activation, AMPA receptor (GluA2 subunit) phosphorylation at serine 880, and subsequent dissociation from the postsynaptic scaffold and endocytosis (21, 23). Given the reduction in mEPSC amplitudes, we tested whether mGluR-dependent LTD was modified in *Nlgn3*^{KO} PCs. In 2- to 3-month-old wild-type mice, application of the group I mGluR agonist DHPG resulted in a persistent reduction of EPSC amplitudes. No depression was observed in *Nlgn3*^{KO} cerebellar slices (Fig. 2D and fig. S2B). This loss in mGluR-LTD may be a consequence of an impaired LTD expression or an occlusion due to constitutive activation. We observed a twofold increase in basal GluA2 phosphorylation at serine 880 in *Nlgn3*^{KO} as compared to the wild-type. Upon DHPG stimulation, phospho-GluA2 levels were increased in wild-type but decreased in *Nlgn3*^{KO} mice, probably due to mGluR-stimulated degradation of the phosphorylated form of GluA2 (Fig. 2E) (24).

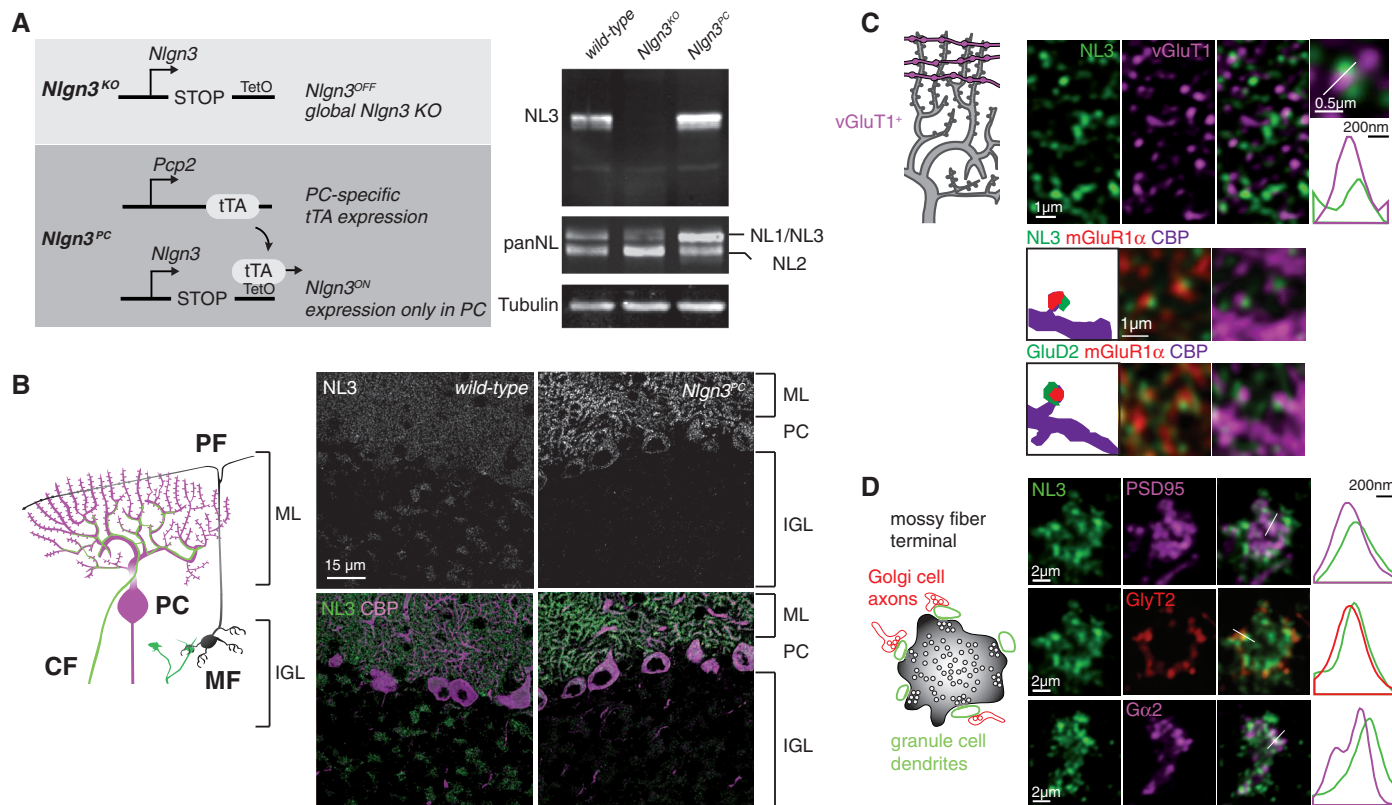


Fig. 1. Cell type-specific synaptic localization of NL3. (A) *Nlgn3*^{KO} (*Nlgn3*^{STOP-tetO}) mice lack NL3 protein expression, and *Nlgn3*^{PC} mice (*Nlgn3*^{STOP-tetO::Pcp2}) show reexpression in cerebellar lysates. (B) Mossy fiber inputs (MF) in the inner granular layer (IGL) are relayed to PCs via parallel fibers (PF). Climbing fibers (CF) form synapses directly onto the proximal PC dendritic arborization. In

Nlgn3^{PC} mice, NL3 immunoreactivity is increased on PC dendrites (calbindin, CBP) and abolished in the IGL. (C) NL3 is apposed to vGluT1⁺ PF boutons and colocalizes with mGluR1 α in CBP⁺ spines, similar to GluA2. (D) In glomeruli, NL3 is detected at PSD95⁺ and GABA-A receptor α 2⁺ synapses and colocalizes with GFP⁺ terminals in *GlyT2*^{GFP} transgenic mice.

The constitutive increase in phospho-GluA2 in the *Nlgn3*^{KO} cerebellum indicated a gain of function in the synaptic plasticity pathway. Parallel fiber LTD is regulated by several postsynaptic

receptors, including GluA2, GluD2, and mGluR1α (21). Using quantitative Western blotting, we discovered a selective increase in the mGluR1α protein in the *Nlgn3*^{KO} cerebellum, whereas the

mRNA level was unchanged (Fig. 3, A and B, and fig. S3; there is a similar mGluR1α protein increase in the thalamus). mGluR2 and mGluR7, two additional metabotropic receptors expressed

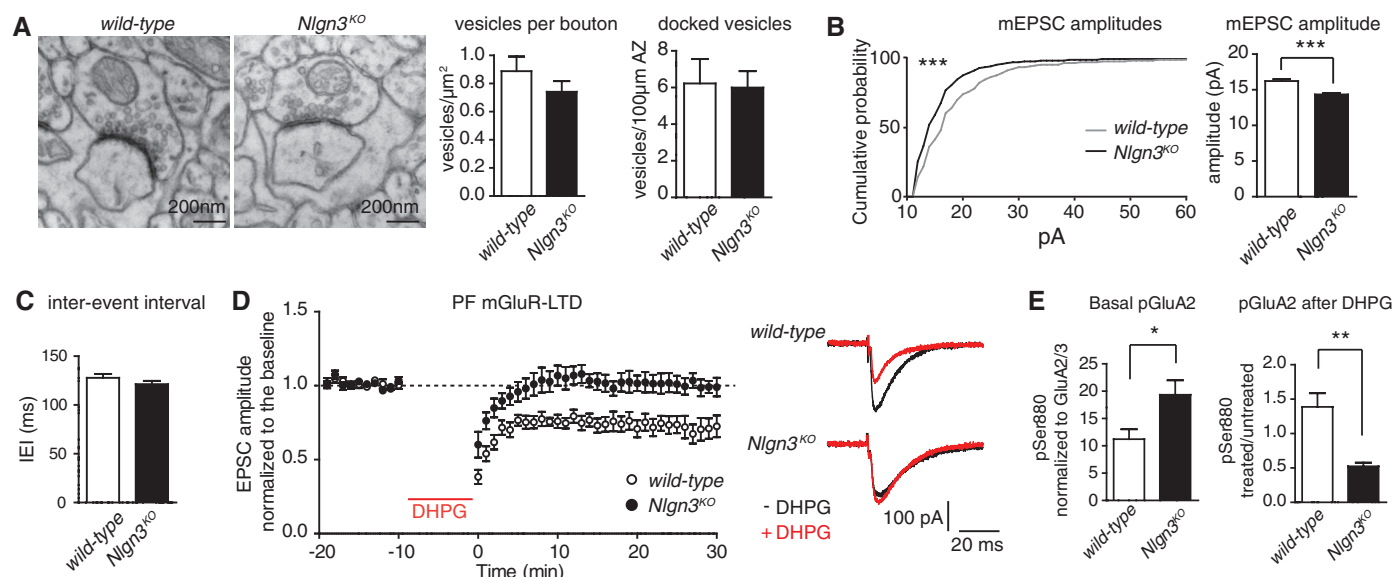


Fig. 2. Occlusion of mGluR-LTD in *Nlgn3*^{KO} mice. (A) PF synaptic ultrastructure in *Nlgn3*^{KO} mice by transmission electron microscopy ($n \geq 4$ animals, 200 synapses per animal). (B) Cumulative distribution of mEPSCs (***P* < 0.001, Kolmogorov-Smirnov test) and mean amplitude ($n = 9$ cells for wild-type and $n = 21$ cells for *Nlgn3*^{KO} mice; ****P* < 0.001, Mann-Whitney test). (C) mEPSC inter-event intervals, paired pulse ratios ($n = 9$ wild-type and $n = 15$ *Nlgn3*^{KO}

cells). (D) mGluR-LTD induced by 10 min of 50 μ M DHPG ($n \geq 8$ cells) and representative traces before (black) and after (red) DHPG stimulation. (E) Quantitative Western blot of basal and DHPG-induced phospho-GluA2 (normalized to GluA2/3 protein level, $n \geq 4$ mice, **P* < 0.03, *t* test). DHPG-induced phosphorylation is expressed as the ratio of treated to untreated samples ($n \geq 4$ mice, ***P* < 0.01, *t* test).

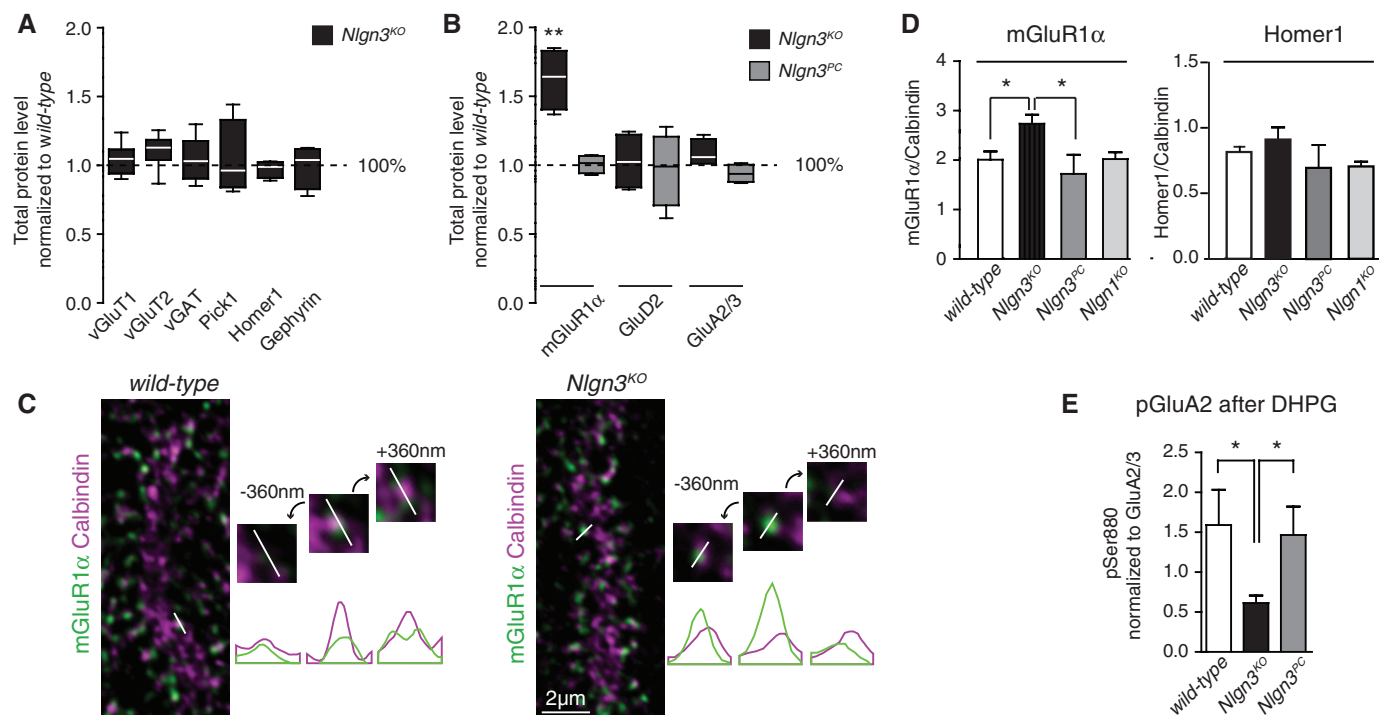


Fig. 3. Elevation of synaptic mGluR1α levels in *Nlgn3*^{KO} mice. (A) Quantitative Western blot. Protein levels were normalized to tubulin and expressed relative to the wild type. (B) Quantitative Western blot of mGluR1α, GluD2, and GluA2/3 levels in the cerebellum (normalized to tubulin and expressed relative to the wild type, $n \geq 4$, ***P* = 0.002, *t* test). (C) Quantitative line scan on cerebellar sections stained with antibodies to mGluR1α, Homer 1, and calbindin.

(D) Line scan intensity ratios of mGluR1α/CBP and Homer1/CBP ($n = 6$ mice, 1200 synapses per genotype; mGluR1α, **P* = 0.04; Homer, *P* = 0.6; *t* test). Reexpression of NL3 in PCs restores mGluR1α level ($n = 4$ mice, *P* = 0.4). There was no difference in *Nlgn1*^{KO} mice ($n = 6$ mice, *P* = 0.9). (E) DHPG-induced phosphorylation of GluA2 expressed as the ratio of treated to untreated samples (normalized to GluA2/3 protein level, $n \geq 5$ mice, **P* < 0.05, *t* test).

in the cerebellum, were unaltered (fig. S3B). Endogenous mGluR1 α and NL3 colocalized in the heads of Purkinje dendritic spines (Fig. 1D). In *Nlgn3^{KO}* mice, synaptic mGluR1 α levels were increased but were unaltered in the *Nlgn1^{KO}* cerebellum (Fig. 3, C and D). This mGluR1 α deregulation was a cell-autonomous consequence of NL3 loss of function, because reexpression of NL3 specifically in PCs (*Nlgn3^{PC}*) reduced the mGluR1 α protein level and its synaptic abundance back to the wild-type level (Fig. 3, B and D). Moreover, DHPG-induced phospho-GluA2 signals were returned to wild-type levels in mice that selectively reexpressed NL3 protein in PCs (*Nlgn3^{PC}*, Fig. 3E).

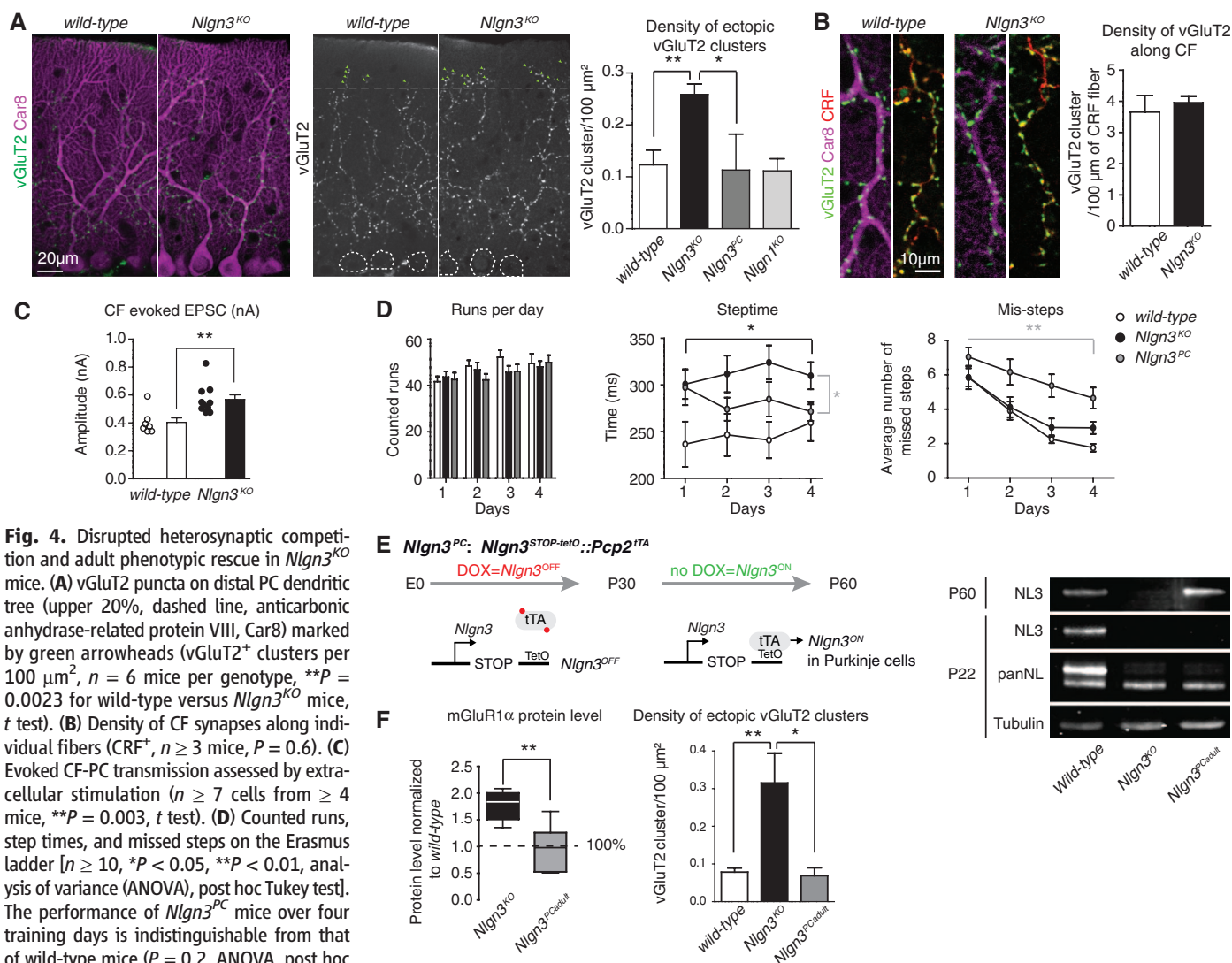
We next examined whether the loss of NL3 results in wiring alterations in the cerebellar network. In the mature cerebellum, each PC is innervated by a single climbing fiber, and synaptic competition between parallel fiber and climbing fiber inputs excludes climbing fiber inputs from

the PC distal dendrites (21, 25). In *Nlgn3^{KO}* cerebella, we observed a significant invasion of vGluT2⁺ terminals into the distal molecular layer (Fig. 4, A and B, and fig. S4A). Ectopic climbing fiber synapses were not observed in *Nlgn1^{KO}* cerebella, and the expression of *Nlgn3* exclusively in PCs was sufficient to suppress ectopic synapse formation in *Nlgn3^{PC}* mice. Synapse density along the entire length of the overshooting climbing fibers was unaltered, resulting in an increased total number of climbing fiber synapses (Fig. 4, A and B, and fig. S4A). Consistent with this observation, evoked climbing fiber transmission was elevated in the *Nlgn3^{KO}* mice (Fig. 4C; there is a normal regression of multi-innervation, fig. S4B).

We used the Erasmus ladder (26) to explore motor coordination in *Nlgn3^{KO}* mice. In this behavioral assay, mice cross a ladder consisting of pressure sensors that measure motor output. Wild-type and *Nlgn3^{KO}* mice completed the same num-

ber of valid runs on the ladder. Step times for *Nlgn3^{KO}* mice were significantly elevated, indicating a perturbation of motor coordination (Fig. 4D). The occurrence of missteps during ladder crossing was unaltered in the *Nlgn3^{KO}* mice and declined similarly as for wild-type mice over several days of training. NL3 expression specifically in PCs rescued the elevated step times progressively after several training days (Fig. 4D). *Nlgn3^{PC}* mice did exhibit a higher number of missteps (Fig. 4D), most likely because the reexpression in PCs exceeds the endogenous NL3 level and results in a gain-of-function phenotype.

The extensive ectopic synapse formation in *Nlgn3^{KO}* mice raises the question of whether such structural defects can be corrected after the completion of development. We used the tetracycline transactivator to temporally control the reexpression of NL3 in PCs of *Nlgn3^{PC}* mice (Fig. 4E). *Nlgn3^{PC}* mice were raised in the presence of doxycycline [transactivator inactive, NL3 expres-



sion off (Fig. 4E)]. At 30 days after birth (P30), doxycycline was removed, permitting the reexpression of NL3 selectively in PCs. Reexpression restored wild-type mGluR1 α protein levels and resulted in the removal of ectopic synapses from the distal PC dendritic tree (Fig. 4F and fig. S4, C and D). Therefore, neurodevelopmental phenotypes and ectopic synapse formation arising from *Nlgn3* deletion can be corrected in the mature cerebellar system.

Our results provide insight into the synaptic pathophysiology of a model of nonsyndromic autism. Phenotypes observed in *Nlgn3*^{KO} mice represent a surprising parallel to the synaptic pathophysiology in *Fmr1* and *Tsc2* mutant mice (27). Like these syndromic autism models, *Nlgn3*^{KO} mice exhibit a deregulation of mGluR-LTD. In the *Fmr1*^{KO} mice, mGluR-LTD in the forebrain and cerebellar neurons is increased, and this phenotype can be suppressed by reduction of group I mGluR activity (5, 28, 29). *Nlgn3*^{KO} mice exhibit an occlusion in mGluR-LTD due to increased mGluR1 α expression, indicating a common core pathway of synaptic dysfunction. mGluR5 antagonists can revert cellular phenotypes in *Fmr1*^{KO} mice and may show therapeutic benefit in some fragile X patients (5, 30). Our work indicates that group I mGluR antagonists hold promise for designing treatment strategies for nonsyndromic autism. Our genetic experiments in *Nlgn3*^{KO} mice reveal that not only functional alterations but also ectopic synapse formation can be reversed, high-

lighting the fact that structural neurodevelopmental phenotypes can be rescued by intervention after the completion of development.

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In Monkeys Making Value-Based Decisions, LIP Neurons Encode Cue Salience and Not Action Value

Marvin L. Leathers^{1,2*} and Carl R. Olson^{1,2}

In monkeys deciding between alternative saccadic eye movements, lateral intraparietal (LIP) neurons representing each saccade fire at a rate proportional to the value of the reward expected upon its completion. This observation has been interpreted as indicating that LIP neurons encode saccadic value and that they mediate value-based decisions between saccades. Here, we show that LIP neurons representing a given saccade fire strongly not only if it will yield a large reward but also if it will incur a large penalty. This finding indicates that LIP neurons are sensitive to the motivational salience of cues. It is compatible neither with the idea that LIP neurons represent action value nor with the idea that value-based decisions take place in LIP neurons.

Each of us makes hundreds of value-based decisions every day. Deciding whether to take a drink of juice or a sip of coffee at breakfast is a process driven by the subjective value of each outcome. So is deciding whether

to go to graduate school or medical school. There has been debate in recent years concerning the neural mechanisms of such decisions. This debate has pitted a goods-based account against an action-based account. The goods-based account holds that limbic areas such as orbitofrontal cortex mediate a choice between goods (juice or coffee) on the basis of their respective values and that the choice is translated into an appropriate motor command (reach for the glass or the cup) in parietal and dorsal frontal cortex (1–3). The action-based account holds that the values of

juice and coffee are computed in orbitofrontal cortex and transmitted to parietal and dorsal frontal cortex, where neurons involved in planning to reach for the glass and the cup become active in proportion to the values of juice and coffee. The decision then evolves through competition between neuronal populations representing the opposed action plans (4–9). The goods-based model is intuitive and fits with the assumption underlying classic behavioral economics that decisions concern anticipated outcomes as distinct from motor plans. However, the action-based model has received apparent support from single-neuron recording studies of the lateral intraparietal area (LIP). In monkeys making value-based decisions between saccade targets, LIP neurons representing each saccade fire early in the trial at a rate proportional to the reward expected upon its completion (10–14). This observation is compatible with the idea that LIP neurons represent action value in the service of an action-based decision process. However, there is another possible interpretation. Emotionally potent stimuli are salient in the sense that they automatically capture attention (15, 16). This is true in particular of stimuli associated with rewards and penalties (17–19). LIP neurons fire at an enhanced rate when attention is directed into their response fields (20). Thus, LIP neurons might fire strongly when a valued target is in the response field simply because the target is motivationally salient (21, 22).

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To distinguish between value-based and salience-based signals in LIP, we used a task incorporating rewards and penalties (23). On each trial, the monkey chose between cues placed in and opposite the neuronal response field (Fig. 1A). Each cue indicated that, if the monkey made an eye movement to its location at the end of the trial, a particular outcome would ensue (Fig. 1B). The possible outcomes were large reward (several drops of water), small reward (one drop of water), small penalty (short period of eccentric fixation), and large penalty (long pe-

riod of eccentric fixation). Confronted with two offers of different value, the monkeys consistently chose the better offer (Fig. 1C and table S2). A large penalty possesses lower value than a small penalty but is emotionally more potent. Consequently, value-based and salience-based models make opposite predictions with regard to the impact of anticipated penalty size on neuronal activity (3, 20, 24). To test the predictions, we collected data from 28 neurons in the right LIP of monkey 1 and 39 neurons in the left LIP of monkey 2.

We first examined trials in which the monkey, offered a choice between a large and a small reward, chose the large reward. Population activity during the cue period (25) was significantly stronger when the cue in the response field predicted a large reward (Fig. 1D, blue fill). The enhancement could have depended on the cue's higher value, its greater motivational salience, or the monkey's decision to look toward the response field. We next examined trials in which the monkey, offered a choice between a large penalty and a small penalty, chose the small

Fig. 1. (A) Sequence of events in a single trial.

The items in each panel were visible to the monkey with the exception of those depicting the response field (RF), gaze location, and saccade. (B) The eight cues possessed different associations in the two monkeys. Large and small water drops indicate large and small rewards, respectively. Large and small lightning bolts indicate large and small penalties. (C) The monkeys nearly always chose optimally. The numbers indicate the percentage of trials on which they chose the better action: either into the RF (blue) or away from it (red). (D) Population firing rate as a function of time during trials with a large-reward cue in the RF and a small-reward cue opposite (blue) or vice versa (red). The monkey chose the large reward. (E) Population firing rate as a function of time during trials with a large-penalty cue in the RF and a small-penalty cue opposite (red) or vice versa (blue). The monkey chose the small penalty. Tick marks indicate 10-ms bins in which the difference between red and blue curves crossed an arbitrary statistical

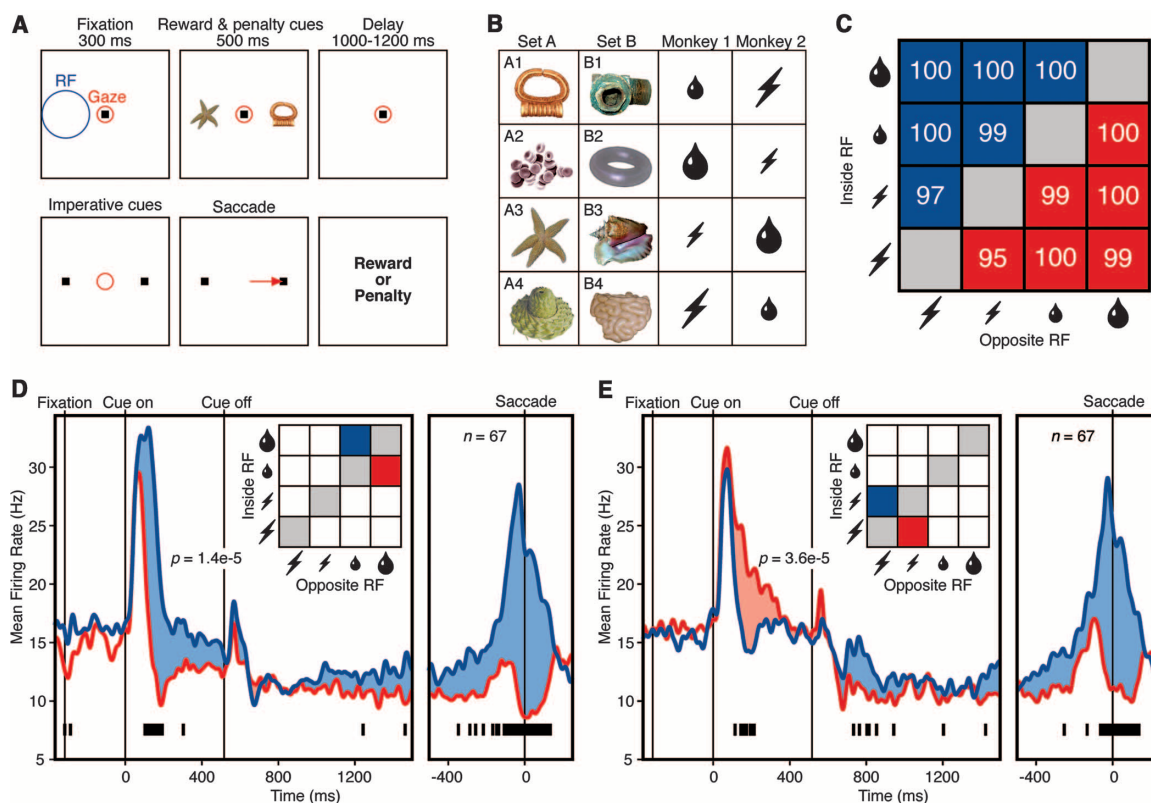
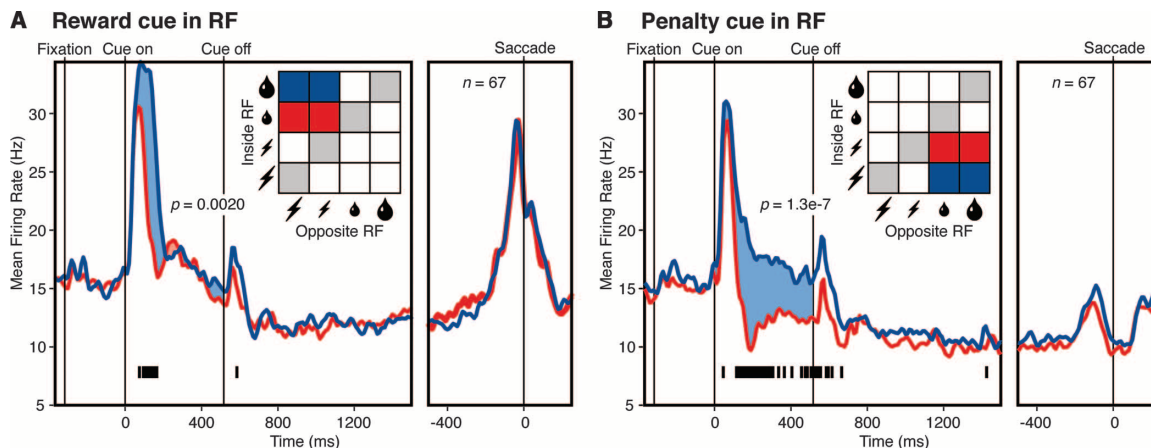


Fig. 2. Population firing rate under selected conditions. The cue in the RF predicted large (blue) or small (red) reward (A) or penalty (B).



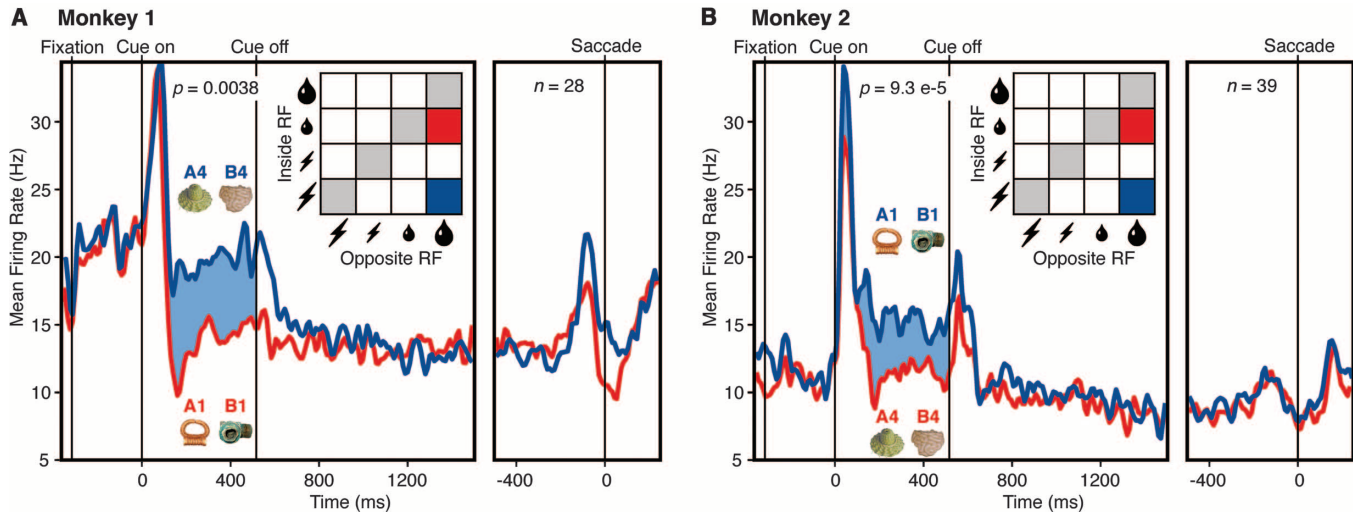


Fig. 3. Population activity when a cue appearing in the RF predicted a large penalty (blue) or a small reward (red). In all trials, the cue opposite the RF predicted a large reward, and the monkey chose it. **(A)** In monkey 1, images A4 and B4 predicted a large penalty, and images A1 and B1 predicted small reward. **(B)** In monkey 2, the contingencies were reversed.

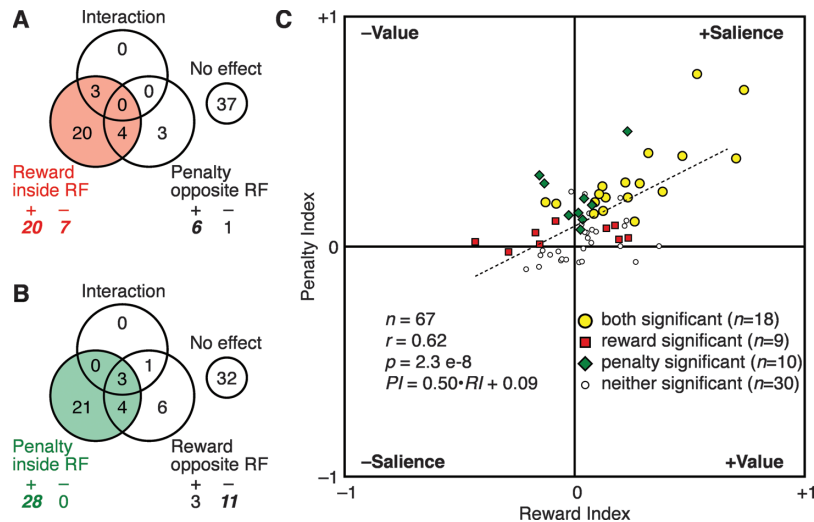


Fig. 4. Individual neurons exhibited significant sensitivity to reward size and penalty size for cues in the RF. **(A)** Counts of neurons exhibiting significant effects of reward size in the RF and penalty size opposite the RF [two-factor analysis of variance (ANOVA), $\alpha = 0.05$]. The predominant outcome was a main effect of reward in the RF (27 neurons, red tinting). **(B)** Counts of neurons exhibiting significant effects of penalty size in the RF and reward size opposite the RF (two-factor ANOVA, $\alpha = 0.05$). The predominant outcome was a main effect of penalty in the RF (28 neurons, green tinting). In **(A)** and **(B)**, “+” and “-” counts indicate numbers of neurons firing more strongly for the larger (+) or smaller (-) predicted outcome; counts in **italic boldface** are significantly greater than those expected by chance (χ^2 test, Yates correction, $\alpha = 0.05$). **(C)** Penalty index versus reward index for all 67 neurons. The quadrants are labeled according to the hypothesis most concordant with the corresponding pattern of activity: +salience, responds more strongly to more-salient stimuli; -salience, responds more strongly to less-salient stimuli; +value, responds more strongly to more-valuable stimuli; -value, responds more strongly to less-valuable stimuli.

penalty. Firing during the cue period was significantly stronger when the cue in the response field predicted a large penalty (Fig. 1E, red fill). This enhancement must have depended on the greater motivational salience of the cue, because its value was low and the monkey decided to look away from it.

To factor out completely any effect of the saccadic decision, we constructed population plots

based on subsets of conditions in which cue value varied but saccade direction did not. Population activity was stronger when a cue in the response field predicted a large as compared with a small reward (Fig. 2A, blue fill) and also when it predicted a large as compared with a small penalty (Fig. 2B, blue fill). The value of the cue opposite the response field exerted only a weak and inconsistent effect (26). This pattern of location spec-

ificity could arise from enhanced spatial attention but not enhanced arousal, vigilance, or general motivation.

Neurons might have responded strongly to large-penalty cues because, by chance, they possessed a high degree of physical salience. Therefore, we gave the images different associations in the two monkeys. Cues predicting a large penalty in monkey 1 predicted a small reward in monkey 2 and vice versa (Fig. 1B). Nevertheless, in each monkey, cues predicting a large penalty elicited stronger firing than cues predicting a small reward (Fig. 3, A and B, blue fill). Thus, neuronal activity depended on the images’ associated outcomes and not their physical attributes.

To characterize reward- and penalty-related activity at the level of individual neurons, we carried out two analyses. One analysis assessed the impact of reward cues inside and penalty cues opposite the response field (Fig. 2A). Main effects of reward size in the response field predominated (red sector of Fig. 4A). A second analysis assessed the impact of penalty cues inside and reward cues opposite the response field (Fig. 2B). Main effects of penalty size in the response field predominated (green sector of Fig. 4B). We next generated for each neuron a reward index and a penalty index based on responses to cues in the response field. Each index ranged from -1 (fired only in response to “small” cue) to +1 (fired only in response to “large” cue). Plotting the penalty against the reward index revealed that they were positively correlated across neurons (27) and that most points fell in the upper right quadrant (Fig. 4C). These points represent neurons whose activity is best explained as encoding the motivational salience of the cues.

That images with greater motivational salience elicit stronger activity in LIP fits with the idea that LIP constitutes a priority map in which

neurons representing a given visual-field location fire at a rate proportional to that location's current draw on attention (20). Neurons in LIP respond strongly to physically salient images (28, 29). Whether they respond strongly to motivationally salient images has been unclear. Cues with greater incentive salience elicit stronger firing (22), but this effect is difficult to disentangle from the encoding of value (30, 31). The current finding that cues with greater aversive salience likewise elicit stronger firing establishes that LIP neurons are indeed sensitive to motivational salience (32).

We have shown that monkeys can make value-based saccadic decisions under circumstances in which the early firing of LIP neurons does not signal action value. This finding jibes with prior reports indicating that neuronal activity in LIP is poorly correlated with target value in a visual foraging task (33), that inactivation of LIP leaves intact the ability of monkeys to make value-based saccadic decisions (34), and that neural activity indicating which reward a subject will choose can develop even if the subject does not yet know which action will be required to obtain it (35, 36). These observations provide collective support for the idea that value-based decisions do not depend on LIP and may instead depend on limbic areas, including orbitofrontal cortex, where neurons signal goods value beginning around 100 ms after cue onset (2, 3).

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Network Resets in Medial Prefrontal Cortex Mark the Onset of Behavioral Uncertainty

Mattias P. Karlsson, Dougal G. R. Tervo, Alla Y. Karpova*

Regions within the prefrontal cortex are thought to process beliefs about the world, but little is known about the circuit dynamics underlying the formation and modification of these beliefs. Using a task that permits dissociation between the activity encoding an animal's internal state and that encoding aspects of behavior, we found that transient increases in the volatility of activity in the rat medial prefrontal cortex accompany periods when an animal's belief is modified after an environmental change. Activity across the majority of sampled neurons underwent marked, abrupt, and coordinated changes when prior belief was abandoned in favor of exploration of alternative strategies. These dynamics reflect network switches to a state of instability, which diminishes over the period of exploration as new stable representations are formed.

The ability of animals to display behavioral flexibility depends on an internal representation of the environment—a framework of beliefs shaped by experience (1–3). How beliefs are encoded remains unclear. Some mod-

els posit gradual updates to the internal representation (4, 5); others invoke abrupt jumps, or resets, in representation (6–9). Such resets may occur after a marked environmental change has been detected, causing prior information to be

discarded. A new internal representation is then constructed by resampling the environment. Indeed, sudden behavioral transitions from the pursuit of a single behavioral option to exploration have been described (10, 11); such findings suggest that prior beliefs can be abandoned in favor of a state of “knowing nothing.”

The medial prefrontal cortex (mPFC) has been implicated in the estimation of relevant environmental statistics that guide the selection of an appropriate behavioral strategy. In primates and rodents, single mPFC neuron activity correlates with the outcomes of previous decisions over diverse time scales (12–16) and can change abruptly in response to global changes of task rules (17, 18). In primates, the mPFC is required for rapid behavioral adaptation to changing action-outcome contingencies (19); in humans, its activity correlates with the volatility of the environment—a parameter known to set the rate of such adaptation

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(5). Activity patterns in the mPFC are consistent with them representing an animal's belief about the environment's governing rules: In essence, the mPFC encodes multiple parameters associated with the structure of a task (20–22), including those that can only be inferred using its internal representation (23). But it remains unclear how ensemble activity in the mPFC changes during a period of uncertainty, when animals have sufficient evidence to reject an existing set of beliefs.

We examined mPFC neural ensemble dynamics in a rat behavioral task designed not only to provide a readout of the transition to exploration but also to enable us to distinguish activity encoding internal beliefs from activity encoding sensation, perception, or behavioral output. In this task, an uncued change in outcome probability causes a state of uncertainty that manifests itself as a decreased resolve to pursue a single behavioral option and an increased exploration of previously rejected choices (Fig. 1). Trial rejection rates often declined transiently after block transitions, prior to a switch in choice preference (Fig. 1B, boxed region in raw data, and Fig. 1D), although some transitions in choice preference happened without transient resampling of both trial types (fig. S1B). Reaction

times for accepted trials increased during these periods of decreased rejection rate, as expected for exploratory bouts (Wilcoxon rank sum test, $P < 0.01$ for 10 trials after versus before acceptance change points).

Unlike deterministic tasks, in which a rule change can be inferred from a single reward omission, detection of change in this task requires gradual evidence accumulation, separating in time the point of abrupt environmental change from the point of awareness. Furthermore, the stochastic nature of action-outcome association invariably leads animals to sample both sides. Thus, even after the animal has detected a change in the environment, analysis of changes in neural activity can be restricted to trials with the same motor output, which is important because changes in motor output can themselves lead to large changes in mPFC activity (24). In addition, at switches in behavioral preference, the local outcome probability will be constant for trials to the same side. This task design therefore permits us to determine whether abrupt dynamics in neural activity are linked to changes in the internal state of the animal.

Within this context, we asked whether representation resets occur in the mPFC when the

animal's confidence in representation of task parameters declines. As in other prefrontal areas, mPFC representation of individual task parameters is distributed across large neuronal networks (21, 25). A sudden reset in the representation of the majority of task-related parameters that should accompany a decision to suddenly abandon old beliefs would likely be revealed as a change in activity that is not only rapid, but also coordinated and widespread across the neuronal ensemble.

We first searched for neurophysiological evidence of abrupt, coordinated, and widespread changes in neural dynamics while blind to the behavioral state of the animal. Retrospectively, we determined whether changes in neural dynamics correlated with behavioral state changes. During each recording session, we found occasions when unexpectedly large changes in neural activity occurred between sequential trials for many of the simultaneously recorded cells (see Fig. 2, A to C, for example and metric description). We refer to the 87 abrupt, coordinated, and widespread changes in population activity, detected across 29 recording sessions, as network transitions. These transitions could not be accounted for by instability in neural recordings (fig. S4), by variance in the animal's

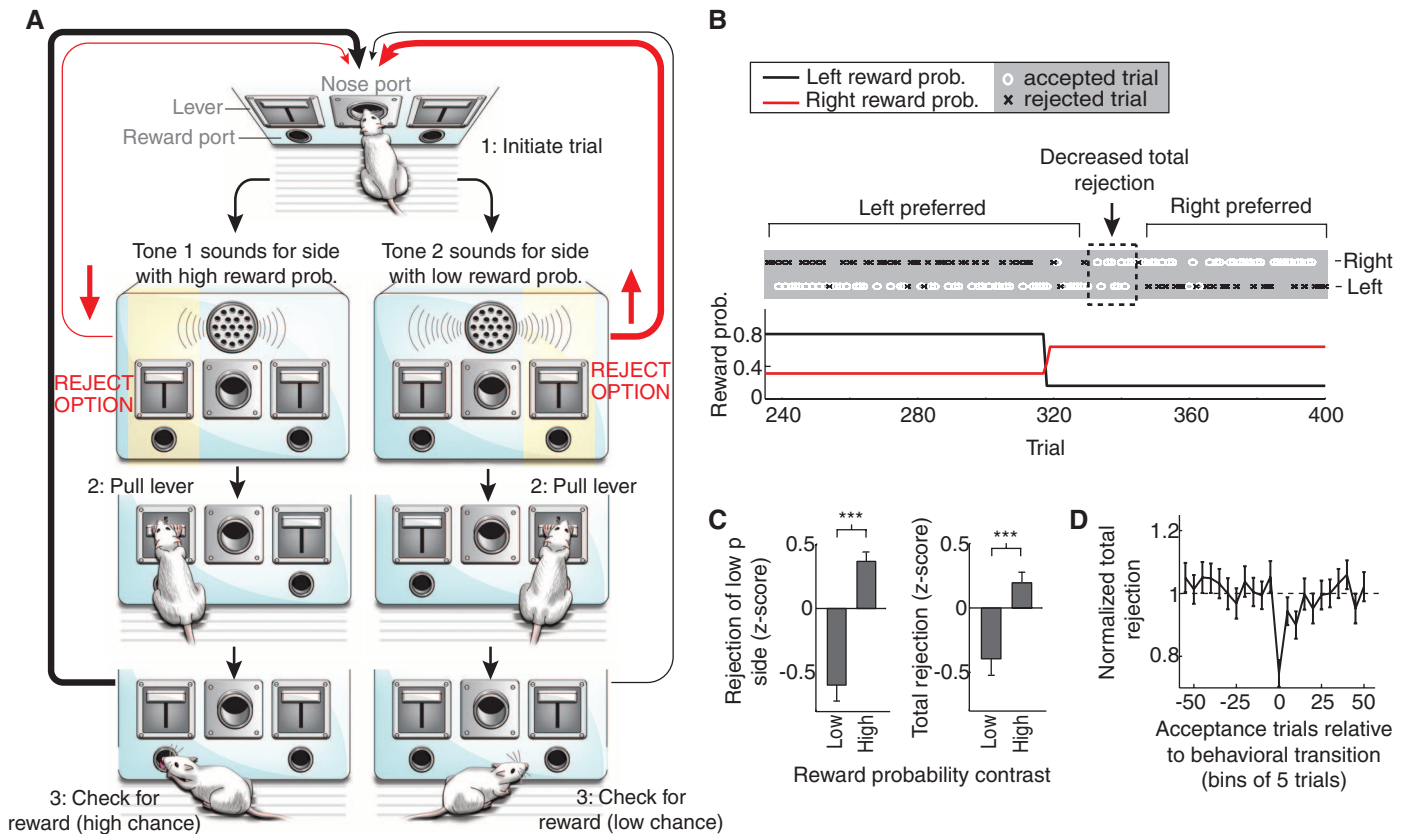


Fig. 1. Rejection rate as a measure of confidence. **(A)** Sequential, tone-cued presentation of two behavioral options, with distinct reward probabilities (between 0.15 and 0.8, changed every 150 to 300 trials). The revealed option can be rejected on each presentation. **(B)** Rejection behavior around a block transition. Note the transient decrease in rejection (boxed region) followed by a commitment to reject the other trial type. **(C)** Standard scores for rejection

rates ($\pm$ SEM) across blocks of trials. Preferential (left) and total (right) rejection rates are higher for high reward probability contrast (high contrast, >0.5 ; low, ≤ 0.5). *** $P < 0.005$, Wilcoxon rank sum test. **(D)** Mean normalized rejection rate ($\pm$ SEM) during transitions in acceptance preference. Note the transient decline in trial rejection around transitions in acceptance. Data in (C) and (D) are from 137 sessions, 12 animals.

spatial trajectory (fig. S5), or by large gaps in the time between trials (fig. S6).

Do these network transitions exhibit the type of dynamics expected when old beliefs are abandoned and task representations are rapidly reset? To determine the abruptness of the activity change across the network, we used a high-dimensional representation of the activity. With the state of the network on each trial represented as a point in this high-dimensional space, we asked whether points for the 10 trials covering the network transition fell into separable clusters, with the network suddenly jumping from one cluster to another within a single trial (Fig. 2D, left panel). Abrupt dynamics were evident for all transitions, which suggests that the associated network-wide activity changes occurred rapidly (Fig. 2D, right panel, and supplementary materials). We next determined the fraction of neurons that displayed firing rate changes above set

thresholds. Around the time of a network transition, 72% of all cells displayed abrupt changes of at least 0.75 of their mean firing rate, and 58% showed changes at least equal to their mean firing rate (Fig. 2E), whereas the average firing rate of the ensemble remained unchanged (Fig. 2F; Wilcoxon rank sum, $Z = 1.16$, not significant).

We next evaluated the temporal relationship between network transitions and rejection behavior. At occurrences of network transitions, the rejection rate typically declined suddenly (Fig. 3, A and B; Wilcoxon rank sum, $Z = 6.99$, $P < 10^{-11}$ for rejection rates in the first versus the second 5 trials of the 10-trial window around the network transitions; see also figs. S7 and S8). Conversely, behavioral segments with negative change in rejection rate were preferentially associated with network transitions for all ranges of acceptance-preference change, reaching significance for me-

dium and high ranges (Fig. 3C). Thus, network transitions do not mark simple changes in behavioral preference or changes in task context. Similarly, a decline in the rejection rate predicted network transitions much more accurately than did local changes in reward rate (fig. S9). The observed abrupt changes in neural activity could not be explained by a sudden change in behavioral output between the two trials flanking the network transition due to the onset of exploratory behavior. With the task design, the manifestation of a decision to resample the previously nonpreferred option had to wait for a variable number of trials, until that option was randomly presented by the computer. In fact, there was no change in local history for a sizable fraction of all network transitions, as the two flanking trials were also consecutive in time (25 of 87; fig. S10); thus, a change in the animal's internal state remained as the only likely explanation.

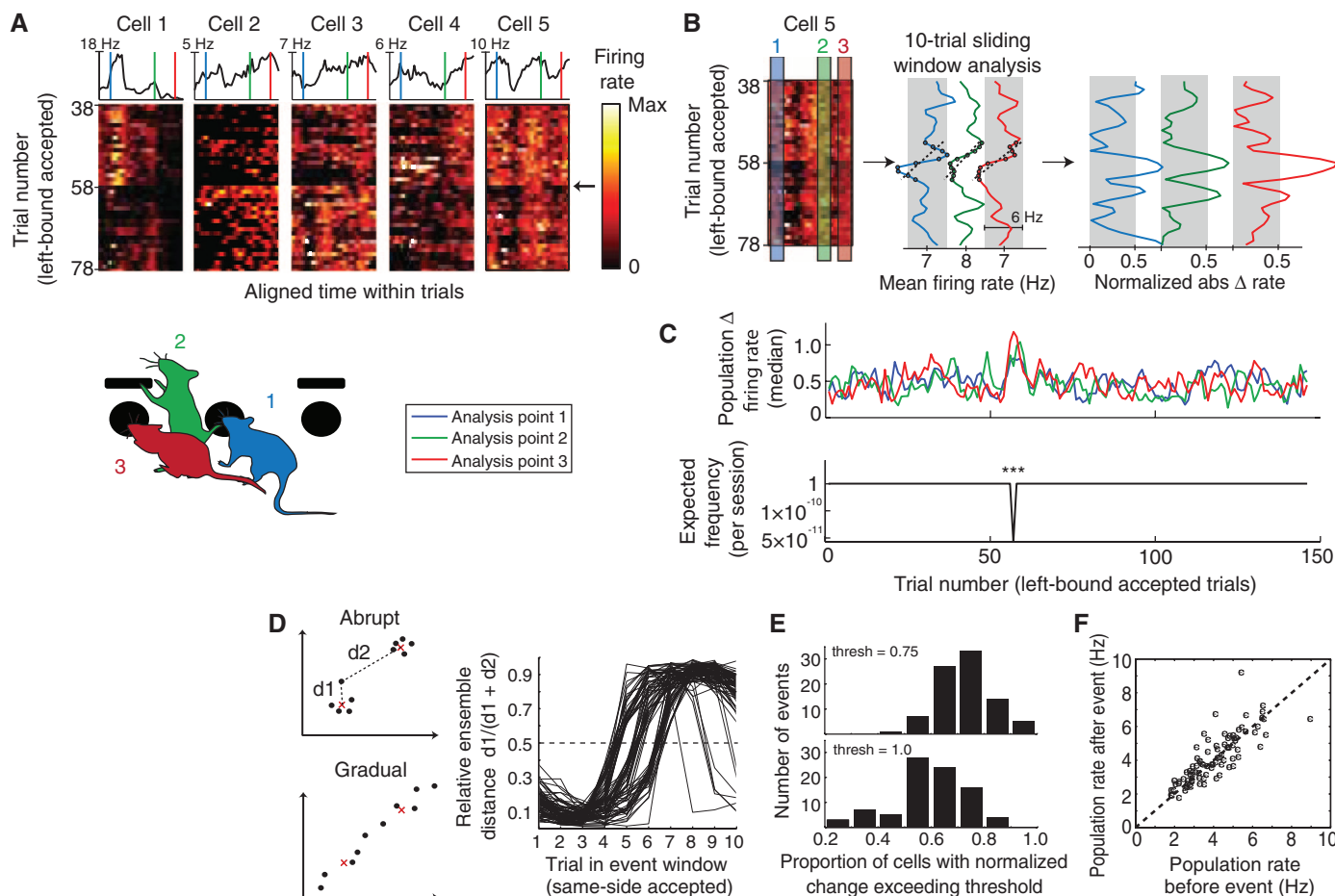


Fig. 2. Network transitions in the mPFC. **(A)** Coordinated activity change for 5 of 15 cells active during left-bound acceptance trials. Time is aligned to the three analysis points (blue, green, and red vertical lines at top, corresponding to colors in cartoon below) when spatial trajectories were stereotyped. Arrow denotes network transition. **(B)** Firing rate changes at each analysis point. Center: Dashed black lines illustrate the slopes of the firing rate; gray rectangles are used for reference of x-axis scaling. Right: Absolute slopes versus trial. **(C)** Detection of network transitions. Top: Absolute firing-rate slope medians across the population of recorded cells [for (A)]. Bottom: Number of

times one would expect to see this level of firing rate change per session by chance. $***P < 0.005$. **(D)** Left: Two scenarios of network state evolution. Upper left: Pre- and post-transition locations form tight separate clusters (red crosses denote centroids of clusters). Lower left: Gradual progression. Right: Relative ensemble distances to pre- and post-transition centroids. **(E)** Network transitions with a given fraction of ensemble displaying firing rate changes of ≥ 0.75 (top) or ≥ 1.0 (bottom) of mean firing rate. **(F)** Mean population rate before and after network transition (5 trials on either side). Points cluster near the identity line. In (D) to (F), data are shown for all 87 transitions.

We also examined the evolution of the dynamics of the network around the time of abrupt transitions. A signature of an upcoming network transition was found in local field potentials

(LFPs) as a transient increase in the power of the high-gamma (65 to 140 Hz) band during the feedback period (analysis time point 3) of the trial preceding the transition (Fig. 4A). No significant

change in LFPs was observable at other analysis time points or in the theta (5 to 10 Hz) or low-gamma (25 to 55 Hz) bands. In contrast to the change in LFPs, which was highly localized in

Fig. 3. Network transitions and the onset of exploration. **(A)** Two network transitions. Top: Activity dynamics of seven cells around the first transition. Dashed line indicates transition; dashed ovals at analysis point 1 mark the transient variable state for each cell. Note synchronized onset of the state change, but variable length of the intermediate state. Middle: Expected frequency for observed median change in firing rate (Fig. 2) during 80 right-bound acceptance trials. Bottom: Trial rejection rates; red circles indicate the 10 peritransition trials. **(B)** Normalized rejection rates (means $\pm$ SEM) for the 10 peritransition trials for all transitions. Network transitions occur between shaded regions. $***P < 0.005$. **(C)** Network transition frequency for nine behavior types (acceptance slope boundaries for equal partitioning: 0, 0.0069, 0.0252, and 0.1019; rejection slope: 0.0728, 0.0108, -0.0114 , and -0.0783). Note strong bias for network transitions during decreases in rejection rate for periods with medium (Z-test for proportions, $Z = 2.50$, $P < 0.013$) and high acceptance change ($Z = 2.43$, $P < 0.016$, Wilcoxon rank sum test). $*P < 0.05$.

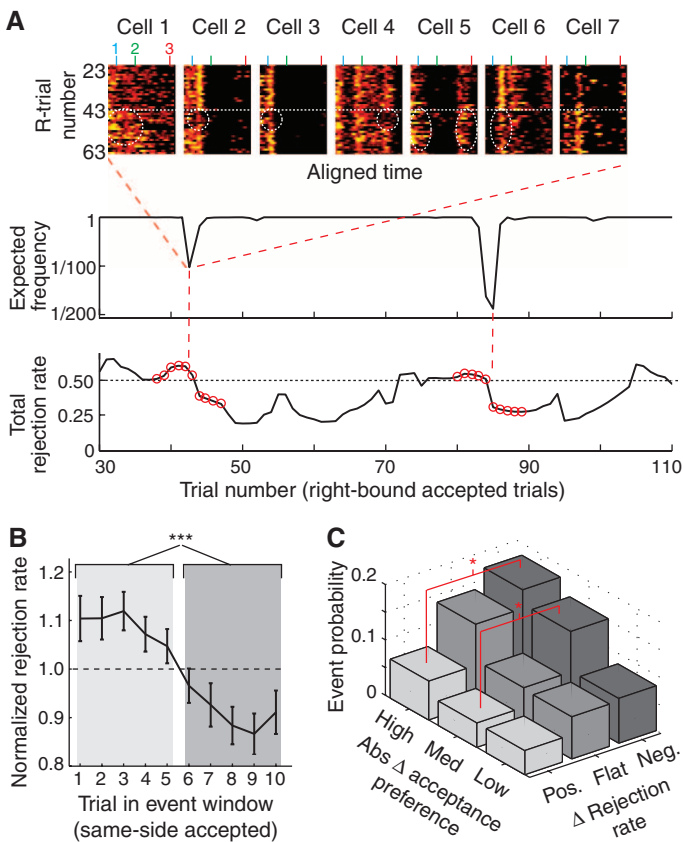
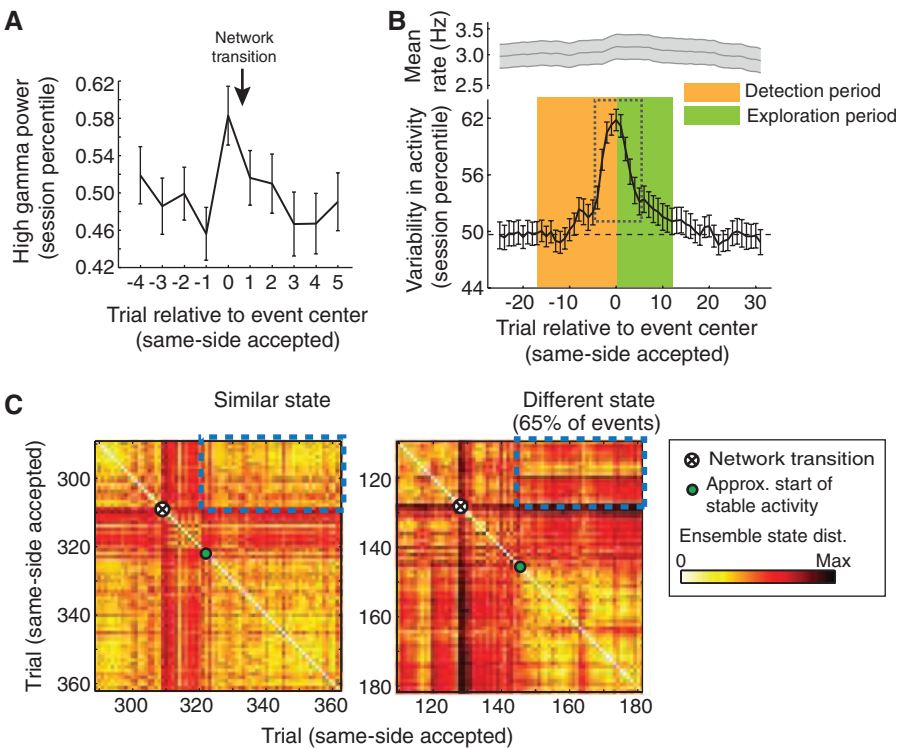


Fig. 4. Network dynamics around transitions. **(A)** High-gamma band (65 to 140 Hz) power of the local field potential at analysis point 3 for 10 peritransition trials. Trials 0 and 1 flank the transition (arrow). **(B)** Trial-to-trial rate variability for 10 trial windows centered on different trials around each detected network transition. Values are average percentiles across all detected network transitions for individual relative window positions ($\pm$ SEM). The dashed box indicates trials (-5 to 5) for which variability is overestimated because of the large network transition. The orange period denotes median time between imposed change and network transition; the green period is the approximate period of exploration based on trial rejection dynamics after network transition. Top: Average ensemble firing rate in the same 10-trial sliding window. **(C)** Similarity matrices for two network transitions from different behavioral sessions derived using Euclidean distance in network state space. The time of network transition and start of new stable activity are indicated along the diagonal.



time, the dynamics of mPFC spiking activity around network transitions were significantly more complex. Average trial-to-trial variability in activity began to increase gradually a few trials after the change in reward probabilities (Fig. 4B; median = 17 trials). After the network transition, trial-to-trial variability remained elevated throughout the exploratory bout (Fig. 4B; 12 trials). To test whether the network returned to the same (or a similar) state after an exploratory bout, we computed a trial-to-trial similarity matrix for the ensemble state (Fig. 4C). Blocks of relative network stability flanked the transient period of increased trial-to-trial variability surrounding the detected network transitions (Fig. 4B). In the minority of cases, the two states were not statistically different, and in a few cases the network likely returned to the same state after the exploratory period (Fig. 4C, left). For most network transitions, however, the subsequent stable network state was significantly different from the preceding state (Fig. 4C, right; 65% of all transitions, Wilcoxon rank sum test comparing pairwise distances within and between states).

Our findings reveal abrupt, coordinated, and widespread changes in neural ensemble activity in the mPFC as animals detect a change in their environment. These observed dynamics in the mPFC are linked to moments when an animal's confidence in its estimates or beliefs about the task environment declines. A previous study suggested that abrupt changes in neural activity represent moments of sudden insight (18). Our analysis did not identify a strong (if any) correlation between network transitions and moments when trial rejection returns to a stable, high level. There are two plausible explanations for this apparent discrepancy: (i) The transitions seen by the earlier study reflect abrupt behavioral changes, or (ii) in a setting of stochastic action-outcome associations, the transition to confidence in the newly established beliefs (and the underlying activity change) is more gradual.

The detection of a highly selective increase in high-gamma power in the mPFC during the feedback period on the trial preceding the network transition suggests that the decision to explore is made at that time. A recent study of

mPFC processing in a task that required monkeys to search through a space of possible hypotheses while searching for a rewarded target (17) described an increase in high-gamma power during the feedback period throughout the search process; the authors interpreted this increase in high-gamma power as a signature of the exploratory period. However, an alternative interpretation that explains both data sets is that high-gamma power in the mPFC is increased whenever the current hypothesis about the world is about to be discarded.

The trigger for abrupt changes in mPFC network dynamics remains unclear. The coordinated nature of the change in mPFC activity raises the possibility that abrupt network reorganization is the result of the action of a neuromodulator, potentially noradrenaline (26, 27). Phasic noradrenaline release in target areas such as the mPFC may trigger network resets whenever rapid behavioral adaptation is warranted in light of unexpected uncertainty (6, 26–28).

After changes in reward contingencies, the temporal link between network dynamics and behavior spans three distinct phases. The slow increase in neural ensemble variability during the first phase is associated with a period when evidence of environmental change is likely to be accumulated (15). The subsequent abrupt network transition correlates with the onset of exploration. The final gradual return of the ensemble variability to baseline is associated with the period of exploratory sampling when new beliefs about task parameters are formed. Although the possibility that the internal representation of the environment's governing rules might also be stored in other brain areas cannot be excluded, the tight temporal link between network dynamics and behavior, taken together with existing lesion and physiological evidence (5, 15, 19, 22, 23), suggests that the mPFC monitors or even directs the evolution of this representation.

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Supplementary Materials

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Materials and Methods

Figs. S1 to S10

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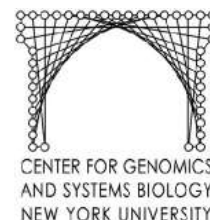
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Letters of application, curriculum vitae, and the names, addresses and telephone numbers of four references should be submitted to: **Carl E. Hock, Ph.D., Associate Dean for Research and Senior Associate Dean, Graduate School of Biomedical Sciences, Chair, Search Committee, c/o Mr. Joshua Lubin, Director of Operations, UMDNJ-School of Osteopathic Medicine, One Medical Center Drive, Academic Center 305, Stratford, NJ 08084 or email: lubinjt@umdnj.edu.** Electronic submissions are encouraged, although paper applications will also be accepted. Interested candidates are encouraged to apply by December 31, 2012. UMDNJ is an Affirmative Action/Equal Opportunity Employer. The Stratford campus is a tobacco-free workplace.



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Neuroscience

Neurodegenerative and Neuropsychiatric Disorders Research:

A Cerebral Career Choice

As people live longer, the incidence of age-related diseases, such as Alzheimer's disease (AD), will also increase. By 2050, the number of people living with AD is expected to triple in the United States alone, from 5.2 million up to 16 million. This potential for increased incidence of AD, as well as other neurodegenerative diseases such as Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS), introduces a critical need for medical breakthroughs that prevent or slow the progression of these diseases. Likewise, neuropsychiatric disorders such as depression and schizophrenia contribute to a substantial proportion of disability among both younger and older individuals. As a result, the field of neuroscience holds a wealth of career opportunities for graduate students and postdoctoral scientists now and in the decades to come. **By Emma Hitt**



Susan Windham-Bannister



Michael Ehlers



Heinz Reichmann

"As our knowledge of brain function explodes, there remains a major need to translate these findings into a meaningful understanding of human brain function in health and disease."

"The math is inescapable," says **Gregory Petsko**, a researcher in neurodegenerative diseases and a professor of biochemistry and chemistry at Brandeis University, in Waltham, Massachusetts. "Unless we find a treatment for the prevention of the major neurodegenerative diseases—including Alzheimer's, Parkinson's, Lou Gehrig's, and so forth—within the next 30 or 40 years, we're cooked." According to Petsko, the increasing problem is fueled by not only the extended lifespan of the population but also by the fact that people are having fewer children. These two factors, says Petsko, will recharacterize the population pyramid, such that diminishing numbers of younger people will be supporting an ever-increasing aging population, changing the pyramid into a column or even an inverted pyramid.

According to **Michael Ehlers**, chief scientific officer for Pfizer Neuroscience, neuroscience-related diseases are "arguably the most significant unmet medical need in the industrialized world." As populations age, the burden of Alzheimer's disease and other dementias grows and is on a trajectory to consume a large percentage of all medical dollars, he says. "Neuropsychiatric diseases represent the largest cause of lost productivity and economic burden—more than all cancers and cardiovascular disease combined—with depression alone being the number one cause of disability. Yet, we have had very few novel therapeutics in this area for many years," he says. "As our knowledge of brain function explodes, there remains a major need to translate these findings into a meaningful understanding of human brain function in health and disease."

Not only is there a need for therapies, but there is also a need for research into the underlying causes of neurodegenerative and psychiatric disorders. "For many of these disorders, we still don't have

effective treatments because the origin of these disorders and ways to target them is still under debate," says **Heinz Reichmann**, president elect of the European Neurological Society. "We are missing a significantly efficacious treatment, and it will be interesting, for example, to determine whether the alpha-synuclein and tau proteins, which contribute to the pathology of Parkinson's and Alzheimer's, respectively, can be targeted to provide more effective treatments," he said.

ADDRESSING IMPORTANT NEEDS

A key need in the field, Petsko says, will be to train physician scientists to translate basic research findings into clinical practice. According to Petsko, the number of Ph.D.s that have been trained in the last 30 years has increased dramatically, but the number of physician scientists has decreased somewhat. He believes that physicians who complete their M.D. training should be encouraged to go into research rather than having to enter clinical practice immediately for financial reasons (i.e., to pay off school loans). "There's nothing else we could do that would make more of an impact," he says. He adds that clinicians who also perform research may be [continued](#)>

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Neuroscience



J. Timothy Greenamyre

more likely than basic researchers to understand how diseases manifest in people. “They are focused on the idea of cures or treatments in a way that many basic researchers are not,” he says. “We need to bring smart people who are broadly trained into the field.”

J. Timothy Greenamyre, professor and vice-chair of neurology at the University of Pittsburgh Medical Center and director of the American Parkinson Disease Association Advanced Center for

Parkinson’s Disease Research at the Pittsburgh Institute for Neurodegenerative Diseases, states that there is a need for students to receive formal training in topics such as “The Neurobiology of Human Disease,” in which they not only learn disease mechanisms, but also interact with individuals affected by these diseases to learn how the disease impacts patients and their families.

Regardless of whether trainees pursue careers in academics or industry, says Greenamyre, most would benefit from formal training in how basic laboratory findings are translated into diagnostics or therapeutics and from learning about topics such as the basics of patents and licenses, the procedures involved in conducting clinical trials, and the U.S. Food and Drug Administration approval process.

Along with adequate training of clinician scientists, research funding must also increase if sufficient headway is to be made in preventing and/or treating the looming increase of age-related disorders. According to Petsko, the total amount of money spent on Alzheimer’s disease research is only about \$500 million per year, and is much less for other neurodegenerative disorders. By contrast, about \$2.4 billion per year is spent on AIDS, which affects a much smaller number of people. “AIDS provides a very valuable lesson, in that it is no longer considered a ‘terrible’ disease, in part, because of advances in biomedical science,” he said. As the population ages, the same trend will hopefully take place in terms of increased funding in Alzheimer’s disease and other neurodegenerative disorders.

According to **David Nutt**, president of the British Neurologic Association and professor at Imperial College London, in the United Kingdom, research opportunities exist in all areas of brain disorder research, but less than 10 percent of research funds are spent on this field even though these disorders cause about 30 percent of all disabilities. He suggests that people in the field should lobby for their discipline.

SPECIFIC OPPORTUNITIES

A recent trend in academia has been for scientists to conduct increasingly collaborative research, and neuroscience-related research is no exception. Thus, graduate students and postdocs who focus on expanding their knowledge base and collaborating with others will create more opportunities for themselves. According to Greenamyre, the Pittsburgh Institute for Neurodegenerative Diseases was conceived and created to eliminate barriers that traditionally impede research on neurodegenerative (or any) disease. “As such, it is composed of about a dozen independent lab groups who share open lab space, and by virtue of both architecture and philosophy, there are no walls between lab groups,” he says. Lab groups from neurology, neurosurgery, [continued](#)

The Massachusetts Neuroscience Consortium — a Collaborative Model

An Interview with Susan Windham-Bannister, president and CEO of the Massachusetts Life Sciences Center

What do you hope to accomplish with the Massachusetts Neuroscience Consortium?

The Massachusetts Neuroscience Consortium is a pioneering new model for accelerating preclinical research, introducing academic researchers to the challenges of targeted research, and facilitating industry-academic partnerships. The pharmaceutical company sponsors will have facilitated access to all of Massachusetts academic and research institutions and will jointly fund projects that leverage the basic, translational, and clinical research expertise resident in the state—the world’s highest density of neuroscience expertise.

According to Congressman Chaka Fattah (D-PA), an advocate for neuroscience research, “This new consortium...is an exciting development for future advances in brain science and medicine. The consortium can provide us with the model for a major national partnership of government, the pharmaceutical industry, leading academic researchers, and medical schools.”

What are the major areas of need in this field in terms of research that you hope to address with the consortium’s efforts?

Neuroscience is a complex discipline in need of both novel therapeutic targets to treat neurological diseases as well as increased understanding of basic mechanisms of function. Significant breakthroughs still elude us in this field, and millions of patients and their families are waiting to hear that we have developed better treatments for diseases such as Alzheimer’s, multiple sclerosis, Parkinson’s, ALS, chronic pain, and others. Interest has also been expressed by consortium members in projects to address the mechanisms of aging, cognition, and synaptic plasticity. Final priorities will be set by the charter members of the consortium.

What opportunities will the consortium and other similar models present for students or postdocs considering a career in neurodegenerative disease research?

We see the Neuroscience Consortium as an opportunity for academic researchers—such as postdocs and graduate students—to build relationships with industry through funded projects and gain exposure to the industry style of research: short-term and results-oriented projects, industry standards for validation, and resources. These opportunities will help young researchers as they determine their next steps in their careers and will facilitate even more effective and productive collaboration between academia and industry.

Given the cost and time involved with the development of new therapies, collaboration is more vital than ever in life sciences research. Traditional approaches to research and drug development have seen a dramatic decline in the number of new therapies moving through the R&D pipelines to patients. Collaboration is essential to accelerating R&D to make a positive impact on curing human disease and improving patient outcomes, especially in complex areas such as neuroscience.

Neuroscience

pharmacology, structural biology, and geriatrics all work together in the same space and on the same diseases.

Another recent effort for collaboration is the Massachusetts Neuroscience Consortium, which was initiated in June 2012. Their goal is to accelerate preclinical research, facilitate industry-academic partnerships, and create “a pioneering new model that is designed to leverage the rich research environment in Massachusetts,” says **Susan Windham-Bannister**, president and CEO of the Massachusetts Life Sciences Center. The consortium brings together seven major industry partners who are willing to collaborate to develop significant advances in major neurological disorders such as Alzheimer’s, Parkinson’s, multiple sclerosis, and neuropathic pain. The companies contributing to the consortium are Abbott, Biogen Idec, EMD Serono, Janssen Research & Development, Merck, Pfizer, and Sunovion Pharmaceuticals (see sidebar on page 144) for more information about this consortium).

For young scientists interested in translational neuroscience, pursuing research in the pharmaceutical industry is particularly rewarding and challenging, says Pfizer’s Ehlers, and “offers the real potential to make discoveries that make medicines.” His advice for young scientists is to think broadly and look outside of traditional career paths. “Training programs for graduate students and postdocs can be quite one-dimensional, exposing trainees to the academic world but little else,” he says. “There is a universe of scientific opportunities outside of the university.”

At Pfizer Neuroscience, Ehlers says, they look specifically for young scientists with a combination of strong quantitative skills and deep knowledge in a specific area, but also with a broad curiosity about all areas of biology. “We also look for people with strong communication skills and an ability to work well collaboratively in teams,” he says.

Graduate students and postdocs interested in a career in this field should also be on the alert for global opportunities, as these diseases will afflict any modernized nation where lifespans are long. In Europe, says Heinz Reichmann, president elect of the European Neurological Society, each country has its own funding sources, both from industry and government, and there are many foundations that support research in this area.

NEW TECHNOLOGIES PROVIDE HOPE

Neurodegenerative diseases are among the most difficult to understand and treat, says **Doug Williams**, executive vice president of research and development (R&D) at Biogen Idec, a company that focuses on neurodegenerative diseases, including multiple sclerosis, Alzheimer’s, and ALS. However, we are living in an era where new technologies such as genomic sequencing and advanced imaging will rapidly increase what we know about these diseases, he says. “By investing in translational medicine, including better neuroimag-

Featured Participants

Alzheimer’s Association
www.alz.org

Atuka
www.atuka.com

Biogen Idec
www.biogenidec.com

Brandeis University
www.brandeis.edu

British Neurological Association
www.bna.org.uk

European Neurological Society
www.ensinfo.org

Massachusetts Life Sciences Center
www.masslifesciences.com

Molecular Neuroimaging
www.mnimaging.com

Pfizer Neuroscience
www.pfizerneuroscience.com

Pittsburgh Institute for Neurodegenerative Diseases
www.neurology.upmc.edu/pind

ing and biomarker strategies, we will be able to make better decisions earlier in clinical development and improve the productivity of our R&D efforts.” This type of investment, he says, “will also enable us to identify which patients may be more likely to benefit from a particular therapy, to determine if a compound is having the intended biological effect on its target, and to detect the progression of a disease even in the absence of new symptoms.” Specific types of scientists who will be most sought after, says Williams, include computational biologists, cell biologists who understand modeling of human diseases, and stem cell biologists.

Jonathan Brotchie is founder and president of Atuka, Inc., a

company with offices based in Canada, the United Kingdom, and China, that provides contract research and consultancy services for the biopharmaceutical industry, specifically to assist larger companies in developing novel therapeutics and diagnostics for Parkinson’s disease.

According to Brotchie, advances may be slowed down not so much by a lack of ideas about drug targets and new therapies, but rather by a lack of understanding of the technologies and methodologies required to develop and validate these ideas. “There is a need to develop better animal models, to recapitulate and predict effects of agents on the molecular pathology of the disease process, and also to develop better imaging and biomarker technologies to assess, as early and precisely as possible, drug effects in clinical studies,” he says.

According to Brotchie, job opportunities are likely to be plentiful at small companies that develop and use cutting-edge technology and capabilities. For example, a PET imaging company, Molecular Neuroimaging, in New Haven, Connecticut, which provides neuroimaging research services to the pharmaceutical and biotech industries, has sprung out of academia to support drug discovery in the field. “These approaches are not available within the pharmaceutical industry and are typically beyond the capabilities of academic groups,” Brotchie says. The picture is very different now than before, he adds, when industry and academia rarely overlapped, but now “convergence, overlap, and cross-fertilization are all part of the environment today for anyone who wants to define a career with a mix of both approaches,” he says.

The challenge now, says Biogen Idec’s Williams, is to go beyond marginal improvements to making transformational changes in how we think about and treat neurodegenerative diseases. “We believe we are at the cusp of a new era when these advances will be possible, but they will require persistence, collaboration, and passion,” he says.

Emma Hitt is a freelance medical and science writer residing in Marietta, Georgia.

DOI: 10.1126/science.opms.r1200124

W UNIVERSITY of WASHINGTON

DIRECTOR WASHINGTON NATIONAL PRIMATE RESEARCH CENTER

The University of Washington seeks candidates for the position of Director of the Washington National Primate Research Center. The Director is responsible for all aspects of the leadership and management of the WaNPRC. The Director is responsible for assuring that the day-to-day operations of the Center are conducted in compliance with all applicable regulations and rules of applicable regulatory agencies and of the University of Washington, and in accord with the mission, guidelines, and reporting requirements of the National Primate Research Center Program as defined by the National Institutes of Health Office of Research Infrastructure Programs.

The successful candidate will (1) possess a significant record of personal success as a scholar and administrator, (2) be the recipient of peer-reviewed biomedical research support, (3) meet the requirements for appointment to an academic department in the University, (4) have experience with, and value of, nonhuman primate research as a model for understanding human health, (5) have a thorough awareness of the national biomedical research environment

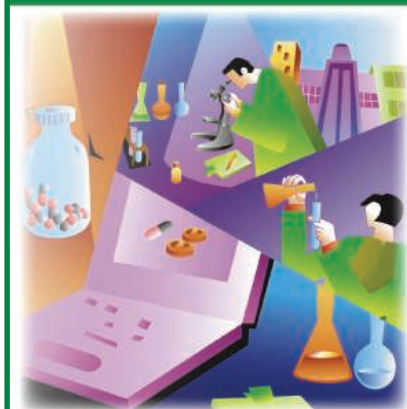
Applicants are required to apply for the position through <http://www.washington.edu/admin/hr/jobs/index.html>, requisition #88569, where you will be asked to submit a letter of interest and complete an online assessment. Priority consideration will be given to those applications received by **November 30, 2012**.

Contact Information:

Director Search Coordinator, Center Program Operations, WaNPRC, Box 357330, University of Washington, Seattle, WA 98195-7330; Email: directorsearch@wanprc.org; Telephone Number: 206-543-0440; Fax Number: 206-616-6771; Primate Center website: www.wanprc.org.

The University of Washington is an Affirmative Action, Equal Opportunity Employer. The University is dedicated to the goal of building a culturally diverse and pluralistic faculty and staff committed to teaching and working in a multicultural environment and strongly encourages applications from women, minorities, individuals with disabilities and covered veterans. All University of Washington faculty engage in teaching, research and service.

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VirginiaTech Invent the Future

College of Science

Neuroscience Faculty

As part of Virginia Tech's expanding presence in emerging interdisciplinary programs and degrees in nanoscience, neuroscience, and computational science, the College of Science seeks to fill tenure-track or tenured faculty positions. These new faculty positions signify a continued University commitment to the importance of interdisciplinary science to help solve major societal challenges in energy, the environment, and health. The successful candidates will have the opportunity to participate in the recently launched College of Science Integrated Science Curriculum (www.science.vt.edu/isc) which has the mission of preparing students as leaders in cutting-edge interdisciplinary science and building strong research and educational partnerships across its eight departments: biological sciences, chemistry, economics, geosciences, mathematics, physics, psychology, and statistics.

Candidates with an emphasis on interdisciplinary research and teaching for a degree-granting program in neuroscience are particularly encouraged to apply. Applicants are expected to have a PhD degree, MD/PhD or DVM/PhD degree. The successful candidate will be expected to establish an internationally recognized research program and be an effective teacher in graduate and undergraduate courses, continue development of scholarly activities and professional capabilities, participate in department, college, and university governance, and pass a criminal background check. Occasional travel to attend professional conferences is required. The candidate will have an opportunity to work closely with other neuroscientists currently at the Virginia Tech Carilion Research Institute (VTCRI). The positions are likely to be at the Assistant Professor level, but outstanding senior candidates will be considered. Please visit www.science.vt.edu and click on the links under Faculty Openings. Consideration of applications will begin as early as **December 1, 2012** and will continue until the positions are filled.

Virginia Tech is an AA/EEO Employer; applications from members of underrepresented groups are especially encouraged.

COLLEGE OF SCIENCE

BIOLOGICAL SCIENCES • CHEMISTRY • ECONOMICS • GEOSCIENCES
MATHEMATICS • PHYSICS • PSYCHOLOGY • STATISTICS

UNIVERSITY OF NEVADA •Reno

The Department of Biology at Nevada seeks two **NEUROBIOLOGISTS** at the assistant professor level, tenure-track. We have two new neuroscience positions in the broad areas of neuron cell biology and neurophysiology. Particular interests are integrative approaches, from cellular levels to plasticity, neural systems, and behavior. Competitive startup funds include support from funded NIH INBRE and COBRE career development grants,

and excellent support facilities. Our faculty are expected to maintain nationally recognized, extramurally funded research programs, to train PhD students, and to participate in undergraduate teaching. The Department has 1200 Biology and Neuroscience majors, 50 graduate students, 24 state-funded faculty, and averages \$4 million/yr in extramural awards. Reno is located in the Sierra Nevada mountains near Lake Tahoe. Reno was recently rated one of the best small cities in the US for outdoor recreation and overall quality of life.

Go to <https://www.unrsearch.com/postings/11531> to submit application materials, including an application letter, CV, research plans, teaching interests, and contact information for three references. Applications received by **November 5, 2012** will receive full consideration.

Equal Employment Opportunity/Affirmative Action. Women and underrepresented groups are encouraged to apply.



Princeton University is committed to the continued expansion and development of neuroscience on its campus. In addition to other outstanding resources, a newly constructed 230,000 square foot building will be opened in late summer 2013 housing state-of-the-art facilities for human brain imaging, cellular and circuit level imaging in non-human species, studies of non-human primates and computation modeling. These new facilities will support the continued growth of neuroscience at Princeton, including the three new positions described below.

Tenured Full Professor Position

The Princeton Neuroscience Institute invites applications for a tenured faculty position at the full or associate professor level, to begin on or after September 2013.

Key selection criteria will be research excellence, originality of science, future impact on the field of neuroscience and related disciplines, and leadership capabilities. Applicants must have an excellent record of research productivity and demonstrate the ability to develop a rigorous research program. We seek applicants pursuing research directions with significant conceptual, theoretical and/or empirical integration across traditional disciplinary boundaries. The successful candidate will join the Neuroscience Institute and may also join a department appropriate to the individual's background and interests, with possibilities including (but not limited to) Psychology, Molecular Biology, Mathematics, Physics, Electrical Engineering and Computer Science. Applicants should be prepared to teach courses both at the undergraduate and graduate levels in neuroscience.

Please submit a curriculum vitae, a brief research description, and contact information for three references at <http://jobs.princeton.edu>, requisition #1200658. Applications will be considered on a rolling basis, and the search will remain open until the position is filled; screening of applications will begin **October 31, 2012**.

Two Tenure Track Assistant Professor Positions

The Princeton Neuroscience Institute invites applications for two tenure track appointments at the assistant professor level to begin on or after September 2013.

Key selection criteria will be research excellence, originality of science, and future impact on the field of neuroscience and related disciplines. We seek applicants pursuing research directions with significant conceptual, theoretical and/or empirical integration across traditional disciplinary boundaries. The successful candidate will join the Neuroscience Institute and may also join a department appropriate to the individual's background and interests, with possibilities including (but not limited to) Psychology, Molecular Biology, Mathematics, Physics, Electrical Engineering and Computer Science. Applicants should be prepared to teach courses both at the undergraduate and graduate levels in neuroscience.

Please submit a curriculum vitae, a brief research description, and contact information for three references at <http://jobs.princeton.edu>, requisition #1200657. Applications will be considered on a rolling basis, and the search will remain open until the position is filled; screening of applications will begin **October 31, 2012**.



FACULTY POSITIONS IN NEUROSCIENCE UNIVERSITY OF KENTUCKY COLLEGE OF MEDICINE

The University of Kentucky College of Medicine invites applications for tenured/tenure-track faculty positions at the ASSISTANT, ASSOCIATE, and FULL PROFESSOR level as part of a college-wide initiative to build upon existing strength in the neurosciences. Three academic units including the Department of Molecular and Cellular Biochemistry, Sanders-Brown Center on Aging, and the Spinal Cord and Brain Injury Research Center have committed support for these positions.

The successful candidates will benefit from a stimulating and collaborative research environment and strong graduate program. Competitive startup funding, salaries, and state-of-the-art facilities are available. Applicants should have a Ph.D. and/or M.D. and multiple years of research experience in neuroscience or a related discipline. Applicants at the Associate or Full rank are expected to direct, while those at the Assistant rank will be expected to develop an internationally recognized, extramurally funded neuroscience research program, to interact with diverse faculty, and contribute to the further development of neuroscience as an area of excellence at the University of Kentucky. Translational programs that encompass basic and/or clinical research and focus on early mechanisms of disease pathogenesis are of special interest.

Interested individuals should visit <http://medicine.mc.uky.edu/neuroscienceref> to access the on-line faculty application and submit a curriculum vitae, statement of research interests and future directions, and the names of at least three references. Inquiries may also be sent to: NeuroscienceFacultySearch@uky.edu. Applications will be reviewed beginning **November 1, 2012**.

The University of Kentucky is an Equal Opportunity Employer and encourages applications from minorities and women.



NORTHWESTERN UNIVERSITY

Neurobiology Assistant Professor

The Department of Neurobiology, in the Weinberg College of Arts and Sciences, seeks to recruit a new tenure-track faculty member at the level of Assistant Professor. Applicants holding a Ph.D. and/or M.D. degree and demonstrating an outstanding record of scientific achievement will be considered. We are interested in individuals whose research addresses fundamental issues in neuroscience and who show significant potential for innovation, scholarship, and commitment to excellence in research and teaching.

Successful candidates will be expected to establish and maintain a high-profile research program attracting substantial extramural funding. The appointee will have access to state-of-the-art life science research support facilities and opportunities to interact with colleagues in the Institute for Complex Systems, Cognitive Neurology and Alzheimer's Disease Center, Center for Reproductive Science, Center for Sleep and Circadian Biology, Robert H. Lurie Comprehensive Cancer Center and an interdepartmental neuroscience graduate program with over 130 faculty.

Applicants will submit (in PDF format) a cover letter, a CV, and a description of research plans. For details on preparing the application, please visit www.neurobiology.northwestern.edu. Please plan to request at least three letters of recommendation. **Applications received by November 1, 2012 will be ensured full consideration. All other inquiries may be directed to Neurobiofacsearch@northwestern.edu.**

AA/EOE. Women and minority applicants are encouraged to apply.

Faculty Positions – Expression of Interest

Australian Regenerative Medicine Institute

About the Australian Regenerative Medicine Institute (ARMI)

Monash University, the Victorian State Government and the Australian Commonwealth Government are investing over \$AUD150m in ARMI, a new research institute for regenerative medicine, focused on unraveling basic mechanisms of the regenerative process and developing clinical applications.

Situated on the Monash University Clayton campus in Melbourne, ARMI is a research Institute within the Faculty of Medicine, Nursing and Health Sciences. Working with multiple centres and disciplines at Monash University and the neighboring institutes and medical centres, ARMI is laying the groundwork for the development of future therapeutic approaches to traumatic injury and degenerative disease, and will pursue rapid commercial transfer for its technologies.

ARMI research groups are located in new, state-of-the-art laboratories with well equipped common facilities creating a framework for a creative, interdisciplinary and highly interactive environment. A key objective of ARMI is to enhance the field of regenerative medicine by promoting the career opportunities for young scientists. As the headquarters of the European Molecular Biology Laboratories (EMBL) Australia Partner Laboratory, ARMI will also include at least six groups operated according to the EMBL research model and philosophy.

About Monash University

Monash is a young, dynamic and internationally recognised university with an established reputation for providing excellence in biomedical research, training and education in campuses around the world. Its Melbourne-based campuses are affiliated with several excellent hospitals. The University is highly regarded for its innovative approach to teaching, research and learning and our graduates are sought after by employers from Australia and overseas. Monash is one of the prestigious Group of Eight universities which are Australia's leading research institutions. Further details about Monash University can be found at www.monash.edu.

The Opportunity

ARMI is now recruiting Faculty staff who wish to establish/relocate their research groups to the Institute's new research facilities.

We seek dynamic, independent scientists with outstanding research track records, demonstrated experience in fields relevant to regeneration, and a desire to work in a multidisciplinary environment. We encourage applicants addressing fundamental questions in developmental, stem cell and regenerative biology, as well as applicants utilising modern systems approaches to apply. Successful candidates will bring a strong track record for attracting independent funding and are expected to initiate and maintain strong research programs synergizing with the Monash research environment and to take active part in collaborative research. Positions are available from mid 2013. The fixed-term appointments will normally be for three years with extension possible upon review.

Enquiries

Professor Nadia Rosenthal, Director, nadia.rosenthal@monash.edu

Professor Peter Currie, Deputy Director, peter.currie@monash.edu

Silvio Tiziani, Chief Operating Officer, silvio.tiziani@monash.edu

Further details are available from www.armi.org

Application

Please submit:

- a cover letter, curriculum vitae and list of publications (indicating ten most significant publications)
- a concise description of research interests and statement of previous research achievements and teaching merits
- a research plan (maximum 8 pages/font 12)
- a financial plan
- a list of at least three reference persons.

Closing date

Tuesday 30 October 2012, 11.55pm Australian Eastern Standard Time

We look forward to receiving your application!

Advertised:

28 September 2012 Australian Eastern Standard Time

An Equal Opportunity Employer



EAS School Chair Search

The Georgia Institute of Technology invites nominations and applications for **Chair of the School of Earth and Atmospheric Sciences** to begin in Fall 2013. We seek an outstanding leader and scientist with an earned doctorate in atmospheric, earth, or space science, or a related discipline. Applicants should be full professors who are strongly committed to interdisciplinary research, undergraduate and graduate education, faculty/staff development, and administrative excellence. Candidates should have demonstrated administrative ability, outstanding research, and the leadership skills to build superior research and education programs.

The School of Earth and Atmospheric Sciences at Georgia Tech is a dynamic and growing department with diverse research activities that focus on the geosphere, atmosphere, and biosphere. The school has 29 tenure track faculty and 17 senior research faculty. It offers M.S. and Ph.D. degrees and has a growing undergraduate program. We seek a chair who can provide leadership for this growth and leverage the assets of Georgia Tech effectively. For further information about faculty research interests, see our web page at <http://www.eas.gatech.edu/>.

Georgia Tech is located on an attractive campus in the heart of Atlanta, a large, diverse, and vibrant city. It is one of the top ranked educational/research institutions in the country and ranked as one of the best places to work. The new chair will have the opportunity to recruit and mentor new faculty and to forge new multidisciplinary interactions with environmental and planetary scientists and engineers at Georgia Tech.

Nominations and applications should be sent in a single PDF file to: EASchair@cos.gatech.edu. Applications should include a cover letter stating interest in the position, a complete curriculum vita describing research, teaching and administrative experience, and the names, addresses, email, and telephone numbers of five references. The search committee anticipates beginning review of applications December 15, 2012; however, applications will be accepted until the position is filled.

Georgia Tech is a unit of the University System of Georgia and an Affirmative Action/Equal Opportunity Employer and requires compliance with the Immigration Control Reform Act of 1986.

IST AUSTRIA LOOKS FOR PROFESSORS AND ASSISTANT PROFESSORS



IST Austria (Institute of Science and Technology Austria) invites applications for Professors and Assistant Professors in physics, chemistry, biology, neuroscience, earth science, mathematics, computer science, and interdisciplinary areas.

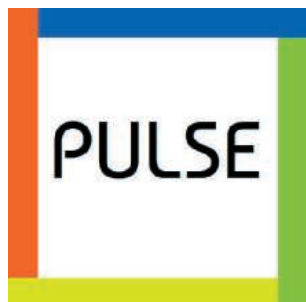
The Institute, which is located on the outskirts of Vienna, is dedicated to basic research and graduate education in the natural and mathematical sciences. IST Austria is committed to become a world-class research center with 1000 scientists and doctoral students by 2026. The working language at IST Austria is English.

The Institute recruits tenured and tenure-track leaders of independent research groups. The successful candidates will receive a substantial annual research budget but are expected to also apply for external research grants.

IST Austria values diversity and is committed to equality. Female researchers are encouraged to apply.

To apply online, please consult:
www.ist.ac.at/professor-applications

Deadline for receiving Assistant Professor applications: **December 1, 2012**
Open call for Professor applications



Vision and Change Leadership Fellows

The Partnership for Undergraduate Life Sciences Education—NSF, NIH/NIGMS, and HHMI—is pleased to announce the 2012 PULSE Vision and Change Leadership Fellows. Over the next year, the forty Leadership Fellows will lead a national conversation to develop effective strategies for the sustained implementation of the *Vision and Change* recommendations. Go to www.pulsecommunity.org to join the conversation.

Karen M. Aguirre, Coastal Carolina University · **Taylor Allen**, Oberlin College · **Judy Awong-Taylor**, Georgia Gwinnett College · **Teresa C. Balser**, University of Florida · **Gita Bangera**, Bellevue College · **Edwin J. Barea-Rodriguez**, University of Texas at San Antonio · **Heather J. Belmont**, Miami Dade College · **Loretta Brancaccio-Taras**, Kingsborough Community College (CUNY) · **Richard A. Cardullo**, University of California-Riverside · **Jonathan R. Cumming**, West Virginia University · **William B. Davis**, Washington State University · **Betsy (Elizabeth) A. Desy**, Southwest Minnesota State University · **Alix D. Dowling Fink**, Longwood University · **Ellen S. Goldey**, Wofford College · **Richard J. Gonzalez**, University of San Diego · **Sharon B. Gusky**, Northwestern Connecticut Community College · **April L. Hill**, University of Richmond · **Thomas Jack**, Dartmouth College · **Nitya P. Jacob**, Oxford College of Emory University · **Michael Ira Kelrick**, Truman State University · **Karen K. Klyczek**, University of Wisconsin-River Falls · **Melanie J. Lee-Brown**, Guilford College · **David J. Marcey**, California Lutheran University · **Kate Marley**, Doane College · **Jenny L. McFarland**, Edmonds Community College · **Kathryn G. Miller**, Washington University in St. Louis · **Marcy P. Osgood**, University of New Mexico · **Joann J. Otto**, Western Washington University · **Pamela A. Pape-Lindstrom**, Everett Community College · **Cynthia B. Peterson**, University of Tennessee · **Jo Anne Powell-Coffman**, Iowa State University · **Todd P. Primm**, Sam Houston State University · **C. Gary Reiness**, Lewis & Clark College · **Sandra L. Romano**, University of the Virgin Islands · **Joel F. Schildbach**, Johns Hopkins University · **Whitney M. Schlegel**, Indiana University Bloomington · **Gina M. Semprebbon**, Bay Path College · **Mary A. Smith**, North Carolina A & T State University · **Akif Uzman**, University of Houston, Downtown · **Gabriele K. Wienhausen**, University of California, San Diego



UNIVERSITY OF OREGON

Faculty Positions in Genomics, Bioinformatics, Statistical Genetics

The Departments of Biology (<http://biology.uoregon.edu>) and Mathematics (<http://math.uoregon.edu>) at the University of Oregon announce a cluster hire of up to three tenure-related faculty positions in Fall 2013. One of these positions may be at the level of Associate or Full Professor with indefinite tenure. These hires are part of an integrated effort to strengthen research and scholarship at the nexus of statistics/mathematics and biology at the University of Oregon, and will serve as a catalyst for future growth in this area. We are broadly interested in recruiting candidates working in areas developing statistical methodology related to the life sciences. Examples of these areas include, but are not limited to, statistical analysis of large data sets, algorithms for analyzing sequence data, and stochastic models for neuroscience, population genomics and molecular evolution. Successful candidates will bolster our emerging strengths in biomathematics, maintain an outstanding research program that focuses on solving core problems in this area, and have a commitment to excellence in teaching. Ph.D. required. Position responsibilities include undergraduate teaching.

Interested persons should apply online to the MATHBIO SEARCH, University of Oregon at <https://www.mathjobs.org/jobs/jobs/4035>. Applicants should submit a cover letter, a curriculum vitae including a publication list, a statement of research accomplishments and future research plans, a description of teaching experience and philosophy, and three letters of recommendation. Ideally the research description and at least one of the letters of recommendation would include descriptions of the statistical/mathematical tools or models used in the applicant's research. To ensure consideration, application materials should be uploaded by **November 15th, 2012**, but the search will remain open until the positions are filled.

Women and minorities are encouraged to apply. The University of Oregon is an Equal Opportunity/Affirmative Action Institution committed to cultural diversity and compliance with the Americans with Disabilities Act, and supportive of the needs of dual career couples. We invite applications from qualified candidates who share our commitment to diversity.



THE UNIVERSITY OF
TOLEDO
1872

Director, School of Biomarker Research & Individualized Medicine (BRIM)

The University of Toledo College of Medicine & Life Sciences invites nominations and applications for the position of Director, School of Biomarker Research & Individualized Medicine (BRIM). Based on the State of Ohio designated Center of Excellence, this new interdisciplinary school is a strategic initiative for The University. The mission of the School of BRIM is to improve the human condition through biomedical innovation resulting from effective multi-disciplinary and inter-professional excellence in biomedical research and education with a focus towards biomarker discovery and personalized medicine; and distinctive entrepreneurship to bring innovations to the community. BRIM investigators represent four colleges (Medicine and Life Sciences; Pharmacy and Pharmaceutical Sciences; Natural Sciences & Mathematics; and Engineering). These 50 highly regarded scientists have strong funding and publication records in biomarker, biosensor and/or individualized medicine research and account for almost \$45 million in extramural funding over the past three years. Further, there are additional investigators who currently collaborate with these investigators and will interface with this school. The University is seeking an individual with the passion and vision to promote the mission of the School of BRIM at the highest level.

The Director will provide visible leadership for BRIM; establish a strong strategy and implementation plan for the research, education, and economic development components of BRIM; provide expertise to BRIM investigators; and work with Departments in multiple colleges to attract and retain strong, funded BRIM investigators. The Director will lead new internal and external collaborations to maximize the potential of BRIM education, research, and economic development.

The successful candidate will have strong track-records in biomarker-related basic, translational and/or clinical research; administration; education; and commercialization. A Ph.D. or M.D. degree and a minimum of three years of senior administrative experience are required. Demonstrated success in research and academic leadership roles at the national level are also required.

Interested candidates should submit a C.V. and a cover letter describing your vision for the establishment of the School of BRIM to: **Director of BRIM Search Advisory Committee, c/o Jackie Hakius, UT College of Medicine & Life Sciences, 2920 Transverse Drive, Mail Stop 1034, Toledo, Ohio 43614 or email: jacalynn.hakius@utoledo.edu.**

The University has an institution-wide commitment to diversity, inclusion, multiculturalism. Applications from women and minority candidates are strongly encouraged. The University of Toledo offers a competitive salary and excellent benefits, and is proudly an Equal Access, Equal Opportunity, Affirmative Action Employer.

Yale University School of Medicine

FACULTY POSITION AT THE JUNIOR OR SENIOR LEVEL

DEPARTMENT OF CELLULAR AND MOLECULAR PHYSIOLOGY

The Department of Cellular and Molecular Physiology is conducting a search for new faculty members at the junior level.

The search seeks candidates whose research connects the properties of molecules to the properties of physiological systems, with particular emphasis on integrative or translational research, or research that makes use of genetic techniques.

Excellent opportunities are available for collaborative research, as well as for graduate and medical student teaching. Candidates must hold a Ph.D., M.D., or equivalent degree. Applicants should include a curriculum vitae, a statement of research interests and goals, and should arrange to have three letters of reference sent. Applications should be emailed to leisa.strohmaier@yale.edu in PDF format.

Application Deadline: **November 16, 2012**

Yale University is an Affirmative Action/Equal Opportunity Employer.

THE UNIVERSITY OF TEXAS

**MD Anderson
Cancer Center**

Making Cancer History®

Assistant/Associate Professor – Cancer Biology

The Department of Cancer Biology has faculty positions available for assistant, associate and full professors, tenure and term tenure-track with demonstrated excellence in molecular and cellular approaches to understanding mechanisms of cancer progression and metastasis. Interests in the areas of cell metabolism, stromal biology and organ fibrosis will be considered. The department and The University of Texas MD Anderson provide an outstanding environment for development of multidisciplinary research careers. Incumbents will establish their own independent research programs and are expected to write grants and manuscripts for publication. Applicants must have a doctoral degree from an accredited university, postdoctoral experience and be eligible to apply for federal grants.

The successful candidate must be able to manage the day-to-day operations in a research laboratory; perform laboratory techniques and use sophisticated laboratory equipment; prepare abstracts for scientific meetings and attend such meetings to exchange scientific data with other researchers; seek funding by writing grants; contribute data for manuscripts to be published in peer-reviewed journals; be able to analyze data using computer software/keyboard, visually proofread all data for accuracy; to manually record in handwriting the results of experiments and test the hypothesis of experiments; communicate both verbally and in writing the results and progress of planned experiments in the laboratory; ensure that safety standards are maintained in the laboratory according to institutional requirements; and read and comprehend research literature and attend seminars to be well informed on latest scientific developments in field of interest.

Interested applicants should submit their current curriculum vitae, research plan, three letters of recommendation and reprints of 2-3 significant publications to: The Department of Cancer Biology, Debra Linzer – Unit 173, The University of Texas MD Anderson Cancer Center, PO Box 301429, Houston, TX 77230-1429.

MD Anderson Cancer Center is an equal opportunity employer and does not discriminate on the basis of race, color, national origin, gender, sexual orientation, age, religion, disability or veteran status except where such distinction is required by law. All positions at The University of Texas MD Anderson Cancer Center are security sensitive and subject to examination of criminal history record information. Smoke-free and drug-free facility.

The Department of Cancer Biology
Debra Linzer – Unit 173
The University of Texas
MD Anderson Cancer Center
PO Box 301429
Houston, TX 77230-1429



International Search for Chair Professors and Distinguished Professors

The University of Macau is a leading higher educational institution in Macao and is making strides towards becoming internationally recognized for its excellence in teaching, research and service to the community. The University is growing rapidly with a number of new strategic initiatives including the relocation to a new campus and the establishment of the largest Residential College system in Asia. The new campus will be 20 times larger than the present one with a projected increase of 40% in student intake and faculty size. English is the University's working language.

We plan to develop a strong team of top-notch scholars to help us realize our vision. Applications are therefore invited from those with excellent academic achievements in the following disciplines:

- Business
- Sciences and Mathematics
- Liberal Arts and Humanities
- Engineering and Technologies
- Education
- Law
- Social Sciences
- Health Sciences

Remuneration and appointment rank offered will be competitive and commensurate with the successful applicants' academic qualification, current position and professional experience. The current local maximum income tax rate is 12% but is effectively around 5% - 7% after various discretionary exemptions.

For details about the above open positions and related information, please visit the following websites:

Job vacancy website: <http://www.umac.mo/vacancy>

University website: <http://www.umac.mo>

Macao government website: <http://www.gov.mo>

Applicants please state the disciplines applied for and quote the reference no.: ACAD/PROF/09/2013 in their application and send it by email or post to the below address on or before 27 October 2012. Review of applications will commence immediately and continue until the positions are filled. Other contact points are

Human Resources Office

University of Macau, Av. Padre Tomás Pereira, Taipa, Macau

Website: <https://isw.umac.mo/recruitment>;

Email: senior.academic@umac.mo

Tel: +853 8397 8593 or +853 8397 8592; Fax: +853 8397 8694

The effective position and salary index are subject to the Personnel Statute of the University of Macau in force. The University of Macau reserves the right not to appoint a candidate.

Applicants with less qualification and experience can be offered lower positions under special circumstances.

Personal data provided by applicants will be kept confidential and used for recruitment purpose only

University of Macau -
An ideal place to pursue your career



The TUM Institute for Advanced Study (TUM-IAS), established in 2005, has become an internationally recognized institution dedicated to fostering advanced innovative research in Science and Technology. It is a cornerstone of TUM's institutional strategy to promote top-level research in the Excellence Initiative by the German federal and state governments. The institute offers an extensive Fellowship program, awarded strictly on the basis of excellence and vision, giving young scientists the time and support they need to develop independent programs while offering established researchers, from industry as well as academia, the freedom to pursue innovative and risky research ideas.

TUM-IAS is looking to fill the position as

Director (m/f)

in succession of the founding Director Professor Patrick Dewilde. The Director is responsible for all affairs of the TUM-IAS. In particular, he/she represents the TUM-IAS in relation to organs, boards, and institutions of TUM and elsewhere, oversees the organization and coordination of the Fellowship Program as well as further activities of the Institute, such as the organization of scientific events. The Director is also responsible for the strategic development of the Institute, in close collaboration with its Board of Trustees. In carrying out these duties, the Director is supported by a professionally competent and highly motivated team.

Candidates must have an outstanding scientific reputation as well as executive experience in a scientific institution, with evidence of their ability to lead a unique research institute where outstanding researchers come together to explore new scientific and technological research problems and so doing contribute to the intellectual life and the innovativeness of the University. Furthermore, they should have extended leadership experience in a university environment, be familiar with interdisciplinary projects, have experience in the evaluation of programs and have developed successful collaborations in an international and interdisciplinary environment.

Candidates should fill the requirements for appointment as Professor at TUM. This senior position requires full time commitment and will be awarded for a term of three to five years. It can be extended to a maximum of further three years and will be compensated at the level of Full Professor in the State of Bavaria. Re-location support for candidates coming from outside Germany will be provided.

As part of the Excellence Initiative of the German federal and state governments, TUM has been pursuing the strategic goal of substantially increasing the diversity of its staff. As an equal opportunity, affirmative action employer, TUM explicitly encourages applications from women and minority groups, as well as others who would bring additional dimensions to the university's research and teaching missions. Preference will be given to disabled candidates presenting essentially the same qualifications. The position is open until filled.

Enquiries and applications should be addressed to:

Prof. Dr. Dr. h.c. mult. Wolfgang A. Herrmann
President
Technische Universität München
praesident@tum.de
Arcisstr. 21
80333 München



The Department of Biology at the University of Nevada, Reno seeks to hire a **GENOME BIOLOGIST** at the assistant professor level, tenure-track. Of particular interest are genomic applications in non-model organisms within the context of behavior, ecology and evolutionary biology. Areas of expertise could include the study of genome structure and function, population and phylogenomics, and epigenetics, including gene-environment interactions. The successful candidate is expected to maintain a nationally recognized, extramurally funded research program, to train PhD students, and to participate in undergraduate teaching. The Biology Department has 1200 majors, 50 graduate students, 24 state-funded faculty, and averages \$4 million/yr in extramural awards. Reno is located in the Sierra Nevada mountains near Lake Tahoe and was recently rated one of the best small cities in the US for outdoor recreation and overall quality of life. Go to **https://www.unrsearch.com/postings/11500** to submit application materials, including an application letter, CV, research plans, teaching interests, and contact information for three references. Applications received by **November 5, 2012** will receive full consideration.

The University of Nevada, Reno is committed to Equal Employment Opportunity/Affirmative Action in recruitment of its students and employees and does not discriminate on the basis of race, color, religion, sex, age, creed, national origin, veteran status, physical or mental disability, and sexual orientation.

Equal Employment Opportunity/Affirmative Action. Women and underrepresented groups are encouraged to apply.



A National Cancer Institute
Designated Cancer Center

Recruiting Scientists in Developmental Cancer Therapeutics

The Hollings Cancer Center and the Medical University of South Carolina (MUSC) announce recruitment opportunities for all levels of faculty with an independent research focus in developmental cancer therapeutics. Areas of interest include new drug targets, drug sensitivity/resistance, mechanism of action, drug metabolism, pharmacogenomics, drug delivery, radiotherapeutics, etc. Faculty will be located in state-of-the-art laboratories in a new building developed to focus on cancer therapeutics. The Hollings Cancer Center is a National Cancer Institute designated cancer center; MUSC holds an NIH-funded Clinical and Translational Science Award. There are multiple complementary shared resources (see: **http://academicdepartments.musc.edu/research/**).

Located on the Atlantic coast of South Carolina, Charleston boasts one of the nation's most historic and picturesque downtown areas, beautiful beaches and international cultural events.

Interested candidates should send their CV, a summary of future research plans, and three references to **www.jobs.musc.edu** and apply to requisition # 048077.

Kenneth D. Tew, Ph.D. D.Sc.
Director Developmental Cancer Therapeutics
Hollings Cancer Center
Medical University of South Carolina
Charleston, SC 29425
tewk@musc.edu

*MUSC is an Equal Opportunity Employer,
promoting workplace diversity.*



SCHOOL OF MEDICINE INDIANA UNIVERSITY

Indiana University School of Medicine – Medical Sciences in Bloomington invites applications for 12-month tenure-track positions in cancer biology using mammalian systems. Emphasis will be given to applicants focusing on cancer epigenetics. The successful applicants will join faculty in the Medical Sciences. Numerous cancer-relevant opportunities exist to develop collaborations with faculty in the College of Arts and Sciences (Biology, Biochemistry, Chemistry), the School of Informatics and Computing in Bloomington (**http://www.soic.indiana.edu/**), the Melvin and Bren Simon Cancer Center (**http://www.cancer.iu.edu/**), the Center for Cancer Systems Biology (**http://motif.bmi.ohio-state.edu/ccsb/**), and the Center for Computational Biology and Bioinformatics in Indianapolis (**http://www.compbio.iupui.edu/**). Successful applicants must have a PhD, MD or MD/PhD, demonstrate a strong potential to establish an externally funded research program, and contribute to the departmental teaching mission at the undergraduate, graduate, or medical level. Additional information may be obtained from: **http://bloomington.medicine.iu.edu**.

Full review of applications will commence **November 1, 2012**, and continue until the position is filled. Applicants should send a single PDF file containing curriculum vitae; statement of research interests and directions; statement of teaching philosophy; and summary of current and anticipated research support to **cancerbi@indiana.edu**. Three letters of reference should be sent under separate cover to **cancerbi@indiana.edu**.

Indiana University is an Equal Opportunity/Affirmative Action Employer. Women and minorities are encouraged to apply.

PRIZES



We are pleased to open nominations
for the 2013 Albany Medical Center
Prize in Medicine and Biomedical Research.

Please visit our website
www.amc.edu/academic/albanyprize/
for nomination information.
Online submission is encouraged.

**Deadline for submission is
December 3, 2012.**

Sêr Cymru

Announcing a £50 Million Research Funding Opportunity in Wales, UK



Llywodraeth Cymru
Welsh Government

www.cymru.gov.uk

The Welsh Government has committed £50 million to the Sêr Cymru programme to attract world-leading scientists to Wales and to support the establishment of collaborative National Research Networks in three 'Grand Challenge' research areas:

- Life sciences and health.
- Low carbon, energy and environment.
- Advanced engineering and materials.

Sêr Cymru aims to increase the value of scientific research to the Welsh economy by developing further an already successful research base, increasing collaboration and expanding facilities and capability in Wales.

The Opportunities

In conjunction with Welsh research institutes, the Welsh Government is seeking Expressions of Interest from:

- World-leading researchers, or Welsh universities, for prestigious Research Chairs with the possibility of additional funding for staff and equipment costs.
- Welsh research institutions to set-up National Research Networks that will grow and shape the future direction of research in Wales.
- High calibre individuals to become Network Directors to lead the National Research Networks and support research excellence in Wales.

Deadline 16th November 2012 for Expressions of Interest.

For further information about the application process and Expressions of Interest pro forma please visit www.wales.gov.uk/sercymru

For this announcement in the medium of Welsh, please visit www.cymru.gov.uk/sercymru

WG16458



The Center for Regenerative Medicine (CRM) at Massachusetts General Hospital and the Harvard Stem Cell Institute invites applications for a tenure-track Assistant Professor position. Outstanding scientists in the field of vertebrate stem cell or regenerative biology will be considered. Candidates with a research program focused on tissue regeneration, plasticity and function and who have a demonstrated interest in application to human disease are especially welcome. Successful candidate(s) will be members of the **Harvard Stem Cell Institute** and faculty of **Harvard University**. Candidates must hold a PhD and/or MD and have a history of innovative, interactive research. Women and minority candidates are urged to apply.

Applicants should send an electronic copy of a (1) letter of interest, (2) research plan, (3) current curriculum vitae and (4) three letters of recommendation to **Dr. David Scadden c/o Miklosik.Mona@mgh.harvard.edu, Center for Regenerative Medicine Search Committee, Massachusetts General Hospital, 185 Cambridge St., CPZN 4265, Boston, MA 02114.**

MGH is an Equal Opportunity/Affirmative Action Employer.

Postdoctoral Training in Gene Regulation

University of Texas Southwestern Medical Center at Dallas

The faculty in the Gene Regulation Core Laboratories of the Cecil H. and Ida Green Center for Reproductive Biology Sciences are currently seeking applicants with a Ph.D. degree for an integrated and dynamic postdoctoral training program in gene regulation. Successful applicants can choose research projects with the faculty listed below.

The research programs in the faculty members' laboratories cover a broad array of topics, including signaling, gene regulation, and genome function, especially in the areas of chromatin, transcription, epigenetics, RNA biology, and nuclear endpoints of cellular signaling pathways. We are interested in a wide variety of model systems and experimental approaches, including biochemistry, molecular biology, structural biology, animal models, genomics, proteomics, bioinformatics, and computational biology.

- **Dr. Xiaoying Bai:** Zebrafish and mammalian models to study the transcriptional mechanisms that regulate the differentiation of hematopoietic stem cells, as well as epigenetic regulation affecting Pol II elongation through chromatin.
- **Dr. W. Lee Kraus:** Signal-regulated transcription in the chromatin environment of the nucleus, with a focus on the estrogen and nuclear NAD⁺ signaling pathways, PARPs, and non-coding RNAs in mammalian biological systems (e.g., hormone signaling, inflammation, ES cell biology, adipogenesis, and metabolism).
- **Dr. Xin Liu:** Interplay between the three-dimensional chromatin organization and RNA polymerase II transcription, focusing on the molecular and biophysical basis of chromatin loop and heterochromatin formation during transcriptional regulation.
- **Dr. Yunsun Nam:** Mechanisms underlying gene regulation by non-coding RNAs, especially those pertinent for development and cancer. The molecular and structural basis of substrate recognition, catalysis, and regulatory mechanisms for the processing of microRNAs.

Candidates should submit a CV or resume, brief statement of interests and accomplishments, and a list of three references in one .pdf or .doc file by e-mail to lee.kraus@utsouthwestern.edu. The subject line should include the terms "Postdoctoral Training in Gene Regulation" and the body of the e-mails should indicate the laboratories of interest. Successful applicants will receive competitive pay and benefits commensurate with the applicant's level of experience.

UT Southwestern provides a dynamic, collaborative, and integrative research and training environment with state-of-the-art facilities.

UT Southwestern is an Equal Opportunity/Affirmative Action Employer.





Birth Defects Research The University of Texas at Austin Dell Pediatric Research Institute

The new Dell Pediatric Research Institute (DPRI; <http://dpri.utexas.edu/>) is a state-of-the-art medical research facility whose mission is to advance understanding of childhood diseases and congenital disorders. **We are inviting applications for tenured or tenure-track faculty positions at all levels. Positions are available for individuals with research programs focused on neurodevelopmental disorders and genetic disorders.** We are interested in candidates who use contemporary approaches to investigate vertebrate and human development and disorders associated with development. We encourage applications from MD/PhD clinician-scientists with expertise in medical genetics/genomics and bioinformatics. Appointments will be made in the appropriate academic unit within the College of Natural Sciences, College of Pharmacy, or School of Engineering. Positions include competitive salary and start-up packages, and laboratory space in DPRI, adjacent to Dell Children's Medical Center just minutes from the UT-Austin campus.

We seek outstanding investigators who will build active research programs, teach effectively at the undergraduate and/or graduate levels, and interface clinically (if appropriate) and scientifically with translational research opportunities at the Dell Children's Medical Center. Successful candidates are eligible for affiliation with various campus-wide research institutes which provide outstanding core research facilities and excellent graduate programs.

Review of applications will begin **October 15, 2012** and continue until positions are filled. Please send a single PDF file containing a letter of application, curriculum vitae, statement of research interests, a one page teaching statement, and names of 3-5 references (who will not be contacted without the consent of the candidate) to:

Dr. Richard Finnell
Dell Pediatric Research Institute
The University of Texas at Austin
1400 Barbara Jordan Blvd.
Austin, TX 78723
email: rfinnell@austin.utexas.edu

The University of Texas is an Equal Opportunity Employer. Qualified women and minorities are encouraged to apply. A background check will be conducted on selected applicants.



FACULTY POSITION Biochemistry & Molecular Genetics

The Department of Biochemistry & Molecular Genetics in the School of Medicine at the University of Virginia (www.virginia.edu/bmg) seeks candidates for a tenure-track faculty position (Assistant or Associate Professor). The successful candidates' research should address contemporary problems in the biology of the nucleus, genomics and computational biology, with emphasis on processes that are important in human disease.

Candidates must hold a Ph.D. and/or M.D. in Biochemistry or a related field and must have demonstrated the capacity to develop and/or maintain a high-quality independent research program. Applicants with outstanding credentials from all fields will be considered. Support will include attractive start-up packages, laboratory space, and an exceptionally interactive research environment.

To apply, visit <https://jobs.virginia.edu> and search on **Posting Number 0610723**. Complete a Candidate Profile online, attach a cover letter, curriculum vitae, and statement of research interest (two pages or less including references). Three letters of recommendation should be submitted electronically, directly to hn6u@virginia.edu with the applicant's name in the subject heading.

For additional information please contact, please contact Helen Norfleet-Shiflett, Department of Biochemistry & Molecular Genetics, via telephone (434) 924-1667 or email hn6u@virginia.edu.

The University of Virginia is an Equal Opportunity/Affirmative employer. Women, minorities, veterans and persons with disabilities are encouraged to apply.



Cambridge, UK

Research Group Leader in Cell Biology/Biophysics

Applications are invited for a tenure-track Group Leader to lead a new research group in the Cell Biology Division of the Laboratory of Molecular Biology. The LMB has an exceptional record of ground-breaking research, excellent resources, and outstanding facilities and workshops.

We seek someone addressing fundamental questions in any area of cell biology, or developing methods relevant to cell biology. Approaches based on neurons, yeast, advanced imaging or biophysical methods are of particular interest, but the primary selection criteria are scientific excellence and potential for synergy with our existing research programmes.

Applicants should hold a PhD and have an excellent publication record with outstanding potential for independent research. Substantial core funding will be available.

Find out more about the LMB's Cell Biology Division at www2.mrc-lmb.cam.ac.uk/research/cb/. Informal enquiries may be made to Sean Munro (sean@mrc-lmb.cam.ac.uk).

The salary for this position will be £40,527 - £43,941 per annum and will be supported by 30 days annual leave entitlement, an optional MRC final salary pension scheme and excellent on-site sports and social facilities.

This position will remain open until it is filled; applications received by 19th November 2012 will be given priority.

Applications should include a covering letter and full CV, an outline of current research interests (1 page) and a proposal for future research (up to 2 pages), along with the names and addresses of three referees who have agreed to be contacted prior to interview. Applications for this post must be made online at http://www.topcareer.jobs/Vacancy/irc67447_2326.aspx. If you do not have access to the Internet, or experience technical difficulties, please contact 01793 867003.

This position is subject to pre-employment screening.

For further information about the MRC visit www.mrc.ac.uk.

The Medical Research Council is an Equal Opportunities Employer

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from the
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sciencecareers.org**

- Job Postings
- Job Alerts
- Resume/CV Database
- Career Advice
- Career Forum

Science Careers

From the journal *Science*





Faculty Position – Bioinformatics

Heal the sick, advance the science, share the knowledge.

The Division of Biomedical Statistics & Informatics in the Department of Health Sciences Research at Mayo Clinic is seeking applications for a full-time, institutionally funded faculty position at the Associate Consultant level in the Bioinformatics Program. The candidate will be responsible for fostering collaboration with investigators from the Center for Individualized Medicine (CIM) and for advancing bioinformatics research activities aligned with the needs of one of the prioritized research areas of CIM: i.e., Biomarker Discovery, Pharmacogenomics and Microbiome. The development and success of the Center for Individualized Medicine is considered a top strategic research priority by Mayo Clinic Leadership.

The candidate should have a PhD in bioinformatics, biology, biophysics, physics or related field and at least 5 years' experience in research. He or she must have strong domain expertise in genomics data preprocessing, data analysis, data interpretation and data integration. The candidate should have demonstrated ability to develop collaboration with investigators and be able to independently and successfully initiate and develop research projects. He or she should be able to lead and give scientific directions to a team of support staff. The preferred candidate will have published research work on Next Generation Sequencing data, and should have experience preparing and submitting proposals for extramural funding, and experience presenting research findings at regional, national or international conferences. He or she must have proficiency in scripting language and/or programming language. Flexibility, creativity and excellent communication skills are required.

This position can be based in Arizona, Florida or Minnesota.

Mayo Clinic is a premier academic medical center with over 3,800 physicians and scientists in a unified multi-campus system. This unique environment brings together the best in patient care, groundbreaking research and innovative medical education. While Mayo Clinic does not offer tenured positions, our policies and practices demonstrate a commitment to ongoing positions. Mayo Clinic offers a highly competitive compensation package with sustained intramural funding, outstanding laboratory facilities, capital equipment funding, technical and computational resources and exceptional benefits.

To apply and learn more, please visit www.mayoclinic.org/scientist-jobs/ and reference **job posting number 6627BR**. Applications should include a cover letter, CV and a statement of research interests. Specific questions related to the posting should be directed to:

Jean-Pierre Kocher, PhD
Associate Professor of Medical Informatics
Mayo Clinic
E-mail: schilbe.jennifer@mayo.edu

Mayo Foundation is an affirmative action and equal opportunity educator and employer. Post-offer/pre-employment drug screening is required.

Scan this QR code with your smartphone and begin your job search.



DEPARTMENT OF MECHANICAL ENGINEERING PROFESSOR AND DEPARTMENT CHAIR

The Department of Mechanical Engineering is accepting applications for the position of Department Chair and Professor, with tenure, starting ideally by September 1, 2013.

The Department currently has 42 primary faculty members (35 tenured or on tenure track), with many holding secondary appointments in other departments and divisions within the College. Undergraduate and graduate enrollments are approximately 500 and 150 respectively. ME faculty also advise almost 100 graduate students enrolled in programs based in other departments and the divisions. Our BS degree in ME allows for optional departmental concentrations in aerospace engineering and manufacturing engineering and college-wide concentrations in energy technologies, nanotechnology, and technology innovation. At the graduate level, the ME Department offers research and professional masters degrees in both mechanical and manufacturing engineering and the PhD in mechanical engineering.

Boston University has made a long-term commitment to the development of the College of Engineering and that commitment is having results. The College is ranked 38th in the nation (USN&WR) and 21st in research funding per faculty. With annual expenditures of over \$8 million, the ME Department's research focus areas include: robotics, control, MEMS and nanotechnology, physical and biomedical acoustics, materials science and engineering, energy and energy systems (including thermofluid sciences), micro-fluidics, biomechanics, advanced manufacturing technologies and computational mechanics. The portfolio is strengthened by the department's affiliation with the Division of Materials Science and Engineering, the Division of Systems Engineering, the Fraunhofer USA Center for Manufacturing Innovation, the Center for Information and Systems Engineering, and the Photonics Center.

The successful candidate will have an earned doctorate and be internationally recognized for research excellence, leadership and scholarship in mechanical engineering or a related discipline. Additionally, s/he will have a sound vision for the future of the Department and the disciplines it represents, the skill to lead and advance a research-oriented department, the ability to both recruit and mentor exceptional junior faculty, and a passion for educational excellence at the undergraduate and graduate levels. The new Chair will be expected to oversee the hiring of multiple new faculty members over the next five years as one aspect of implementing their vision for the future. The Department is particularly interested in a leader who will further strengthen the graduate program through an environment that fosters interdisciplinary research and industry collaboration. More information on the Department can be found at www.bu.edu/me/

Applications will be considered until the search committee has identified a suitable list of viable candidates. It is unlikely that applications received after January 15, 2013 will be considered. Applicants should submit an electronic dossier that includes a cover letter, curriculum vitae, and the names and addresses of at least six references to: mechairsearch@bu.edu

Boston University is an Equal Opportunity and Affirmative Action Employer.

MONTEREY BAY AQUARIUM RESEARCH INSTITUTE

2013 POSTDOCTORAL FELLOWSHIP PROGRAM

Applications for the postdoctoral fellowship program at the Monterey Bay Aquarium Research Institute (MBARI) are currently being accepted. MBARI is dedicated to the development of state-of-the-art instrumentation, systems, and methods for scientific research in the oceans. Ongoing programs in marine robotics, ocean physics, chemistry, geology, and biology as well as information management and ocean instrumentation research and development exist at MBARI. Located in Moss Landing, California at the head of Monterey Bay, MBARI enjoys convenient access to diverse oceanographic environments. The institute operates research vessels equipped with remotely operated vehicles, autonomous underwater vehicles, and diverse oceanographic equipment. In addition, MBARI operates the MARS seafloor cable observatory. MBARI is a non-profit oceanographic research institute supported by the David and Lucile Packard Foundation.

Offers will be made to candidates from the fields of biological, chemical, physical oceanography, marine geology, and ocean engineering. Candidates must be awarded the Ph.D. degree prior to commencing the two-year appointment and start during the 2013 calendar year. Applicants are encouraged to communicate with potential research sponsors at MBARI for guidance on project feasibility, relevance to ongoing research projects, and resource availability (http://www.mbari.org/about/postdoc_mentors.htm).

Application deadline: Wednesday, December 12, 2012

Selected candidates will be contacted in early March 2013.

Application requirements:

1. Curriculum vitae
2. At least three professional letters of recommendation
3. Succinct statement of the applicant's doctoral research
4. Potential research goals at MBARI
5. Supplemental Information online form (http://www.mbari.org/oed/jobs/forms/postdoc_form_2013.htm)

Competitive compensation and benefits package.

MBARI considers all applicants for employment without regard to race, color, religion, sex, national origin, disability, or veteran status.

Address your application materials to:

MBARI, Human Resources

Job code: Postdocs-2013

7700 Sandholdt Road, Moss Landing, CA 95039-9644

Submit by e-mail to: jobs_postdocs@mbari.org (preferred), by mail, or fax to (831) 775-1620.



EOE • MBARI Welcomes Diversity

POSITIONS OPEN

CELL BIOLOGIST

The Biology Department of Franklin & Marshall College invites applications for a two-year **VISITING ASSISTANT PROFESSOR** position in cell biology, beginning July 2013 (pending administrative approval). Candidates should have the Ph.D., demonstrated strengths in teaching and research, and broad interests in cell biology. Teaching responsibilities will include lecture and laboratory sections of a sophomore-level core course in cell biology, and an upper level elective in the candidate's area of specialization. Franklin & Marshall is a small (enrollment 2,400), highly selective coeducational liberal arts college with a tradition of excellence in science and student research. In 2012, the Howard Hughes Medical Institute awarded the College a substantial grant to encourage the development of future leaders in translational research and public health through the College's innovative partnership with the Clinic for Special Children.

The successful candidate will have the opportunity to participate in our interdisciplinary major programs, including Biochemistry & Molecular Biology and Biological Foundations of Behavior (neuroscience and animal behavior). Applicants should arrange to have letters sent from three referees, and should submit a cover letter, curriculum vitae, plans for actively engaging undergraduates through teaching and research, teaching evaluations (if available), and undergraduate and graduate transcripts. Electronic applications will not be accepted. Priority will be given to completed applications received by November 16, 2012. Send applications to: **Dr. David Roberts, Department of Biology, Franklin & Marshall College, P.O. Box 3003, Lancaster, PA, 17604. Telephone: 717-291-4118; fax: 717-358-4548; e-mail: janice.kaufman@fandm.edu; website: <http://www.fandm.edu/biology>.**

Franklin & Marshall College is committed to having an inclusive campus community, and as an Equal Opportunity Employer, does not discriminate in its hiring or employment practices on the basis of gender, race or ethnicity, color, national origin, religion, age, disability, family or marital status, or sexual orientation.

ASSISTANT PROFESSOR POSITION

College of Liberal Arts and Sciences
University of Illinois at Chicago

The Department of Biological Sciences in the College of Liberal Arts and Sciences at the University of Illinois at Chicago (UIC) invites applications for a tenure-track **ASSISTANT PROFESSOR** position in Neurobiology to start August 16, 2013. Final authorization of the position is subject to availability of state funding. Located in the heart of Chicago, UIC is one of the nation's leading urban research universities. Numerous opportunities exist for collaborative research in biological sciences across disciplines at UIC and with colleagues and institutions throughout the Chicago region.

The successful candidate will establish a vigorous, externally funded research program, teach undergraduate neurobiology courses and participate in the graduate neuroscience training program. Applicants working in all areas of neurobiology are encouraged to apply. Neurogenetic and advanced imaging approaches to cellular or systems neurobiology are of particular interest. This individual will join a diverse department that investigates a wide breadth of topics in biology, supported by excellent facilities and resources. Candidates must have a Ph.D. or equivalent, significant postdoctoral experience, a demonstrated record of research accomplishments, and teaching experience.

For fullest consideration, candidates must complete an online application and submit a curriculum vitae, research and teaching statements, and the names and e-mail addresses of three references at **website: <https://jobs.uic.edu>** by December 1, 2012.

Women and members of traditionally underrepresented minority groups are strongly encouraged to apply. The University of Illinois is an Affirmative Action, Equal Opportunity Employer.



Memorial Sloan-Kettering
Cancer Center

The Best Cancer Care. Anywhere.

FACULTY POSITION

Structural Biology Program

The Structural Biology Program of the Sloan-Kettering Institute (www.ski.edu) invites applications for a tenure-track faculty position at the Assistant Member level (equivalent to Assistant Professor). We are interested in individuals who have an outstanding record of research accomplishments. Areas of interest include x-ray crystallography, NMR spectroscopy, EM and optical imaging, as well as the interface of structural, chemical and computational biology. Faculty will be eligible to hold graduate school appointments in the Gerstner Sloan-Kettering Graduate School of Biomedical Sciences, the Weill Cornell Graduate School of Medical Sciences, as well as the Tri-Institutional MD/PhD Training Program.

The deadline for applications is November 1, 2012. Interested candidates should visit <http://facultysearch.ski.edu> to access the on-line faculty application. Please visit the site as soon as possible, as it contains important information on the required application materials, including deadlines for submission of letters of reference.

Informal inquiries may be sent to **Julie Kwan** at kwanj@mskcc.org or to **Dr. Nikola Pavletich, Chair, Structural Biology Program** at pavletin@mskcc.org.

facultysearch.ski.edu

MSKCC is an equal opportunity and affirmative action employer committed to diversity and inclusion in all aspects of recruiting and employment. All qualified individuals are encouraged to apply.

2013 Novartis Prizes for Immunology



The 2013 Novartis Prizes, one for Basic Immunology and one for Clinical Immunology are awarded for outstanding achievements in the understanding of immunology and major immunological discoveries that resulted in therapeutic applications in such fields as autoimmune and inflammatory diseases, transplantation, cancer immunotherapy, infectious diseases, dermatology and asthma. For more information, please visit www.novartisimmunologyprizes.org

The next Novartis Prizes for Immunology will be awarded at the XVth International Congress of Immunology in Milan, Italy in a special Award Ceremony on August 23, 2013.

Each prize is endowed with SFr 100,000.

Nominations in English should include a summary of the major research activities in up to 2 pages, curriculum vitae, and a bibliography containing the most relevant publications pertaining to the candidate's nomination.

The deadline for entries is 25 January 2013.

Nominations should be sent electronically to: novartisprizes@gmail.com
Dr. Jan E. de Vries, Secretary of the Novartis Prizes for Immunology, P.O. Box 2501,
La Jolla, CA, 92037, USA.



Jury: Hidde Ploegh (Chair), Charles Dinarello, Tadimitsu Kishimoto, Bernard Malissen, Diane Mathis, Anne O'Garra, Jan de Vries.

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FACULTY POSITIONS IN NEUROSCIENCE

As part of the 2nd Century Initiative at Georgia State University, the Neuroscience Institute (website: <http://neuroscience.gsu.edu>) and the Departments of Biology (website: <http://biology.gsu.edu>) and Psychology (website: <http://www2.gsu.edu/~wwwpsy>) are seeking two senior faculty members in the area of Neurogenomics and the Molecular Basis of Behavior pending budgetary approval. Particular attention will be paid to applicants engaged in research on genetic, genomic, or epigenetic processes, molecular mechanisms of behavior, or behavior genetics. Researchers using any organism including invertebrates, vertebrate laboratory animals, nonhuman primates, and humans are welcome to apply. This research will complement and extend GSU's current strengths in systems, computational, and behavioral neuroscience. Hires will be made at the rank of **ASSOCIATE** or **FULL PROFESSOR**, and candidates are expected to have federal funding for their research.

Applications should include a full curriculum vitae, names and contact information for three references, and a letter of research interest. Applications can be submitted either electronically in pdf format to e-mail: neurogenomics@gsu.edu or in hard copy to: **Chair of the Neuroscience Search, Neuroscience Institute, Georgia State University, PO Box 5030, Atlanta, GA 30302.**

In addition to these positions, Georgia State University's Second Century Initiative (2CI) is supporting separate cluster-hiring initiatives in primate social cognition, neuroimaging, neuroethics, obesity, bioinformatics, therapeutics, and other selected areas. For more information about the 2CI initiatives, see website: <http://www.gsu.edu/secondcentury/>.

Review of applications will begin immediately and will continue until the positions are filled.

For more information contact the search committee chair, Dr. Kim Huhman (e-mail: khuhman@gsu.edu).

Georgia State University, a Research University of the University System of Georgia, is an Affirmative Action/Equal Employment Opportunity employer. Employment is contingent upon background verification.

POSTDOCTORAL POSITION in the Molecular Biology of Female Reproductive Systems University of Texas Southwestern Medical Center at Dallas

Seeking individuals with a Ph.D. or M.D. degree and relevant research experience for a postdoctoral position in the laboratory of Dr. W. Lee Kraus. The Kraus laboratory has a number of exciting ongoing projects related to the molecular role of estrogen signaling, inflammation, and poly(ADP-ribose) polymerases (PARPs) in various aspects of female reproductive biology, including parturition, endometriosis, and mammary carcinogenesis.

Candidates should have demonstrated experience in molecular biology and mouse genetic models, as well as an ability to work independently and creatively. Experience with genomic approaches (e.g., RNA-seq, ChIP-seq) is a plus. Candidates should submit a curriculum vitae or resume, brief statement of interests and accomplishments, and a list of three references in one .pdf or .doc file by e-mail: lee.kraus@utsouthwestern.edu. The subject line should include the terms "Molecular Biology of Female Reproductive Systems postdoctoral Application". Successful applicants will receive competitive pay and benefits commensurate with the applicant's level of experience.

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Georgia Southern University's Department of Biology invites applications for **TENURE-TRACK ASSISTANT PROFESSOR POSITIONS** in Fisheries Biology, Parasitology, and Physiology and two non-tenure-track Lecturer appointments with expertise to teach genetics and/or microbiology. All positions require teaching, service, and a terminal degree. The tenure-track positions also include research responsibilities. The full text advertisements, including information about the department, faculty, and the complete position announcements with all qualifications and application instructions, are available at website: www.bio.gorgiasouthern.edu. Screening of applications begins November 5, 2012, and continues until the positions are filled. Georgia is an open records state. Georgia Southern is an Affirmative Action/Equal Opportunity institution. Individuals who need reasonable accommodations under the ADA to participate in the search process should contact the Associate Provost.

TENURE-TRACK POSITION Department of Chemical Engineering and Materials Science University of California, Davis

Applications are invited for a faculty position at the **ASSISTANT PROFESSOR** level in chemical engineering. All areas of expertise will be considered, and candidates with an interest in the area of catalysis are especially encouraged to apply. The candidate should have a strong research record with the potential and commitment to become a leader in the field. Commitment to undergraduate and graduate education is essential. A Ph.D. in chemical engineering or a related discipline is required. Consult website: <http://chms.engineering.ucdavis.edu/> for our on-line application procedure and requirements. The position is open until filled; but to assure full consideration, applications should be submitted no later than October 19, 2012, for a start date of July 1, 2013.

UC Davis is an Affirmative Action/Equal Opportunity Employer, and is dedicated to recruiting a diverse faculty community. We welcome all qualified applicants to apply, including women, minorities, individuals with disabilities, and veterans.

ASSISTANT PROFESSOR OF BIOLOGY

The Department of Biology at Barnard College, Columbia University, seeks a full-time, tenure-track Assistant Professor starting July 2013, to participate in undergraduate teaching and establish an active, externally funded research program. We are interested in candidates who are broadly trained and address questions at the physiological and/or whole-organism level.

Teaching responsibilities include an advanced lecture, laboratory, and seminar course in the candidate's area of specialization, and participation in the core genetics course. Ph.D. and postdoctoral experience is required; teaching experience is desirable.

Applicants should send curriculum vitae, research and teaching statements, three representative publications and three letters of recommendation electronically to e-mail: biologyjob@barnard.edu. Review of applications will begin November 1, 2012.

Barnard College is an Equal Opportunity Employer. Women and members of underrepresented minorities are encouraged to apply.

Three (3) Tenure-Track Faculty Positions in Biology: One position in Cellular/Molecular and two positions in Animal Physiology within the Biology Department at York University (Toronto, ON, Canada) all at the **ASSISTANT PROFESSOR** level. Applications must be received by December 31, 2012 for a start date of July 1, 2013. Please consult website: <http://www.yorku.ca/acadjobs> for more information. York University is an Affirmative Action Employer. The Affirmative Action Program can be found at the website above or a copy can be obtained by calling the affirmative action office at telephone: 416-736-5713. All qualified candidates are encouraged to apply; however, Canadian citizens and Permanent Residents will be given priority.

LOYOLA MARYMOUNT UNIVERSITY Frank R. Seaver College of Science and Engineering

Tenure-track Faculty Position in Microbiology or Plant Biology, with expertise in Population/Ecological Genetics: The Department of Biology at Loyola Marymount University (LMU) invites applications for a tenure-track position at the rank of **ASSISTANT PROFESSOR**, beginning fall 2013. Individuals with a Ph.D. in Biology or related fields are encouraged to apply. The candidate is expected to teach upper division courses in Plant Biology or Microbiology, lower division courses in Biology that include population genetics and/or molecular genetics, and courses in his or her specialty area. In addition to excellent teaching of a diverse undergraduate student body, the candidate is expected to establish an active research program that will include undergraduate students. Synergy in research and teaching is enhanced by opportunities such as the CURES Center for Urban Resilience and the construction of the new Life Sciences Building to begin in 2013. Situated adjacent to the Ballona Wetlands in Los Angeles, LMU is in a culturally and ecologically diverse environment. It is within two hour's drive of beaches, deserts, and mountains; the California Channel Islands are also close by. Please send a letter of application, curriculum vitae, selected publications, a statement of teaching philosophy within an institution such as LMU, a description of research accomplishments and goals, and arrange for three letters of recommendation to be sent to: **Dr. Urbinati, Department of Biology, Loyola Marymount University, 1 LMU Drive MS 8220, Los Angeles, CA 90045-2659.** Review of applications will commence 29 October 2012 and continue until the position is filled. As a comprehensive Catholic, Jesuit/Marymount University, LMU seeks professionally outstanding applicants who value its mission and share its commitment to academic excellence, the education of the whole person, and the building of a just society. The successful candidate will be committed to supporting and enhancing a culturally rich and diverse learning environment. LMU is an Equal Opportunity Institution actively working to promote an intercultural learning community. Women and minorities are encouraged to apply.

FACULTY POSITION in Cell Biology University of Maryland Baltimore County (UMBC)

The Department of Biological Sciences at University of Maryland Baltimore County (UMBC) invites applications for a tenure-track **ASSISTANT PROFESSOR** position in eukaryotic cell biology, especially as it relates to neurobiology, to complement existing departmental strengths in neuroscience and cell and developmental biology, and to interact with faculty whose interests range from genomics and molecular genetics to ecology, evolution, and behavior. The successful applicant will have a Ph.D. in a relevant field of biological sciences, postdoctoral experience and a strong publication record and is expected to establish a vigorous, externally funded research program, supervise doctoral-level graduate students, and teach at the undergraduate and graduate levels.

Applicants should submit a cover letter, curriculum vitae, summary of current research and future plans, and a statement of teaching interests and philosophy e-mail: biosearch@umbc.edu in a single PDF in the above order. The applicant should also arrange to have three letters of reference in PDF format, sent to e-mail: biosearch@umbc.edu. For full consideration, applications should be submitted by November 15, 2012 but will be accepted until the position is filled.

UMBC is a medium-sized research university in the Baltimore-Washington, D.C. area, whose combined excellence in research and outstanding educational programs have earned recognition by U.S. News and World Report as the "#1 Up-and-Coming National University" for four years running. For information about the Department of Biological Sciences and its graduate programs, visit website: <http://www.umbc.edu/biosci/>.

The University of Maryland Baltimore County is an Equal Opportunity/Affirmative Action Employer. UMBC values gender, ethnic, and racial diversity; women, members of ethnic minority groups, and individuals with disabilities are strongly encouraged to apply. UMBC is the recipient of an NSF ADVANCE Institutional Transformation Award to increase the participation of women in academic careers.



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Activin A	CD95 / sFas Ligand	sFlt-4/ Fc Chimera	sIL-6 Receptor	MIP-1 β / CCL4	RPTP β
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apo-SAA	Desmopressin	GDNF	IL-17	NFAT-1	TACI
Apolipoprotein A-1	Disulfide Oxidoreductase	GLP-1	IL-17B	beta-NGF	TARC
Apolipoprotein E2	E-selectin	Glucagon	IL-17D	NOGGIN	TC-PTP
Apolipoprotein E3	ECGF	Goserelin	IL-17E	NOV	TECK
Apolipoprotein E4	EGF	GM-CSF	IL-17F	NP-1	TFF2
APRIL	Elafin/SKALP	GPBB	IL-19	NT-1/BCSF-3	TGF- α
Artemin	EMAP-II	GRO α	IL-20	NT-3	TGF- β 1
ATF2	ENA-78	GRO β	IL-22	NT-4	TGF- β 2
Aurora A	Endostatin	GRO γ	IL-31	Ocreotide	TGF- β 3
Aurora B	Enteropeptidase	GRO/MGSA	Insulin	Oncostatin M	Thymosin α 1
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BAFF Receptor	Eotaxin-2	Growth Hormone BP	JE	OTOR	sTIE-2/Fc Chimera
BCA-1 / BLC / CXCL13	Eotaxin-3 (TSC)	GST-p21/WAF-1	JNK2a1	Oxytocin	TL-1A
BCMA	EPHB2	HB-EGF	JNK2a2	p38- α	TNF- α
BD-1	EPHB4	HCC-1	KC / CXCL1	Parathyroid Hormone	TNF- β
BD-2	Eptifibatide	HGF	KGF	PDGF-AA	sTNFR1
BD-3	Erk-2	Histidyl-tRNA synthetase	L-asparaginase	PDGF-AB	sTNFR2
BDNF	Erythropoietin (EPO)	Histrelin	LAG-1	PDGF-BB	TPO
Bivalirudin	Exodus-2	HRG1- β 1	LALF Peptide	Persephin	TRAIL/Apo2L
BMP-2	Fas Ligand	I-309	LAR-PTP	PF-4	sTRAIL R-1 (DR4)
BMP-4	Fas Receptor	I-TAC	LC-1	PIGF-1	sTRAIL R-2 (DR5)
BMP-7	FGF-1 (acidic)	IFN- α	LBP	PIGF-2	TSH
BMP-13	FGF-2 (basic)	IFN- α A	LD-78 β	PKA α -subunit	TSLP
sBMPR-1A	FGF-4	IFN- α 2a	LDH	PKC- α	TWEAK
Brain Natriuretic Protein	FGF-5	IFN- α 2b	LEC/NCC-4	PKC- γ	TWEAK Receptor
BRAK	FGF-6	IFN- β	Leptin	Pleiotrophin	Urokinase
Breast Tumor Antigen	FGF-7/ KGF	IFN- γ	LIGHT	PLGF-1	VEGF121
C5a	FGF-8	IFN-Omega	LIX	Polymyxin B (PMB)	VEGF145
C5L2 Peptide	FGF-9	IGF-I	LKM	PRAS40	VEGF165
C-10	FGF-10	IGF-II	LL-37	PRL-1	VEGF-C
C-Reactive Protein	FGF-16	proIGF-II	Lymphotactin	PRL-2	VEGF-C I525
C-Src	FGF-17	IGFBP-1	sLYVE-1	PRL-3	EG-VEGF
Calbindin D-9K	FGF-18	IGFBP-2	M-CSF	Prokineticin-2	VEGF-E
Calbindin D-28K	FGF-19	IGFBP-3	MCP-1 (MCAF)	Prolactin	HB-VEGF-E
Calbindin D-29K	FGF-20	IGFBP-4	MCP-2	Protirelin	sVEGFR-1
Calmodulin	sFGFR-1 (IIIc) / Fc Chimera	IGFBP-4	MCP-3	PTHrP	sVEGFR-2
Calcitonin Acetate	sFGFR-2 (IIIc) / Fc Chimera	IGFBP-5	MCP-4	PTP1B	sVEGFR-3
Carbonic Anhydrase III	sFGFR-3 / Fc Chimera	IGFBP-6	MCP-5	PTP-IA2	WISP-1
Carcino-embryonic Antigen	sFGFR-4 / Fc Chimera	IGFBP-7	MDC (67 a.a.)	PTP-MEG2	WISP-2
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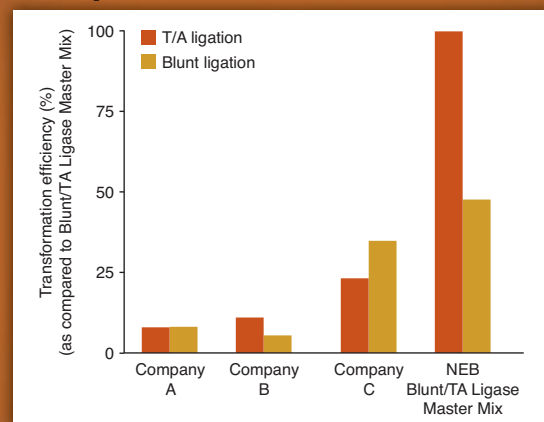
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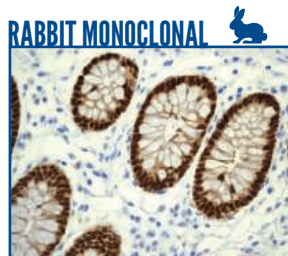
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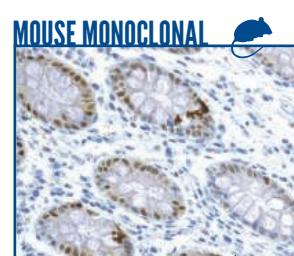
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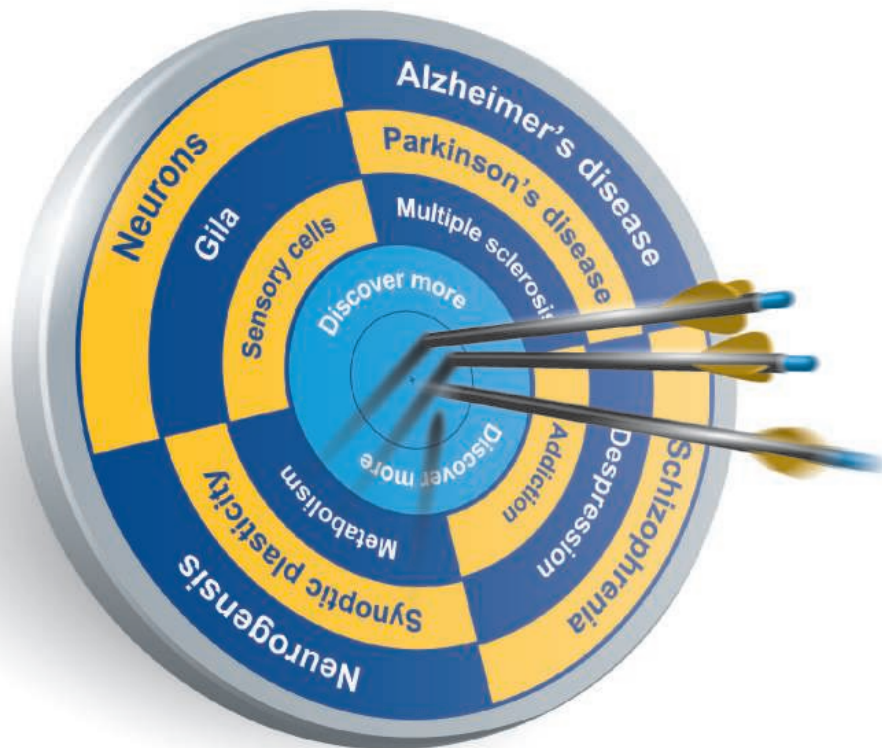
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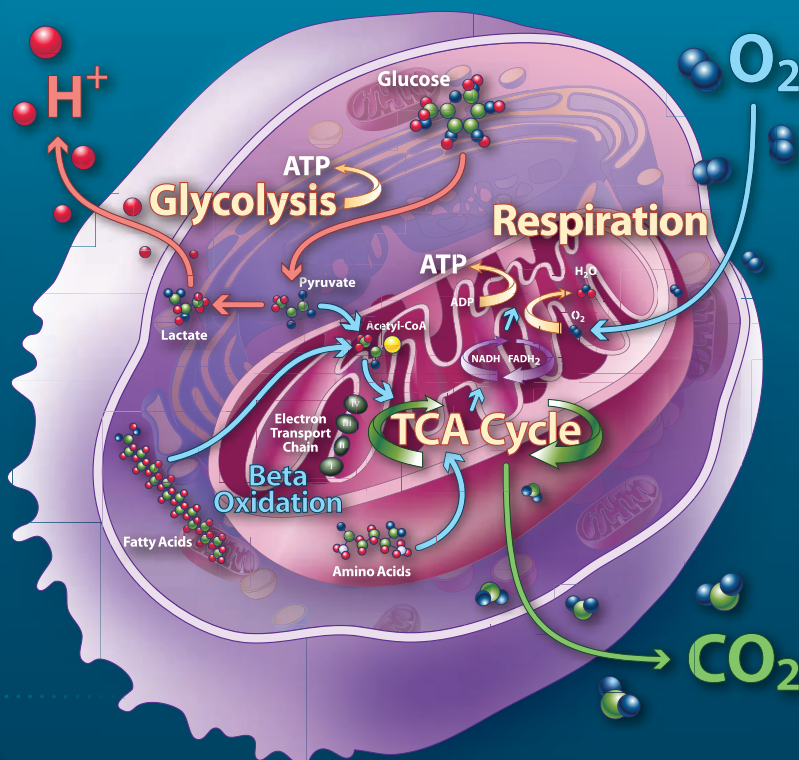


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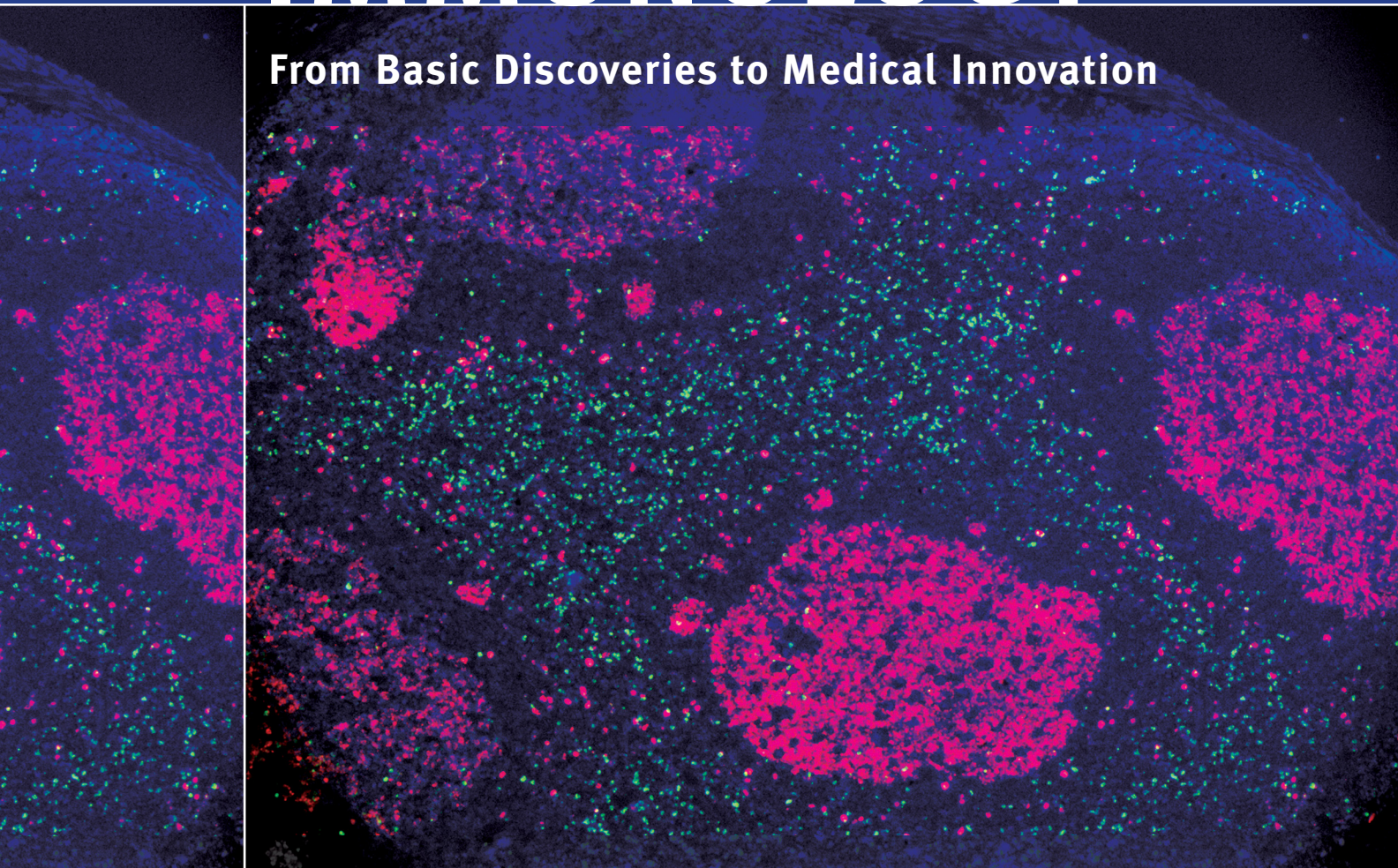
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PROGRESS IN IMMUNOLOGY

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DEEPENING OUR UNDERSTANDING IN IMMUNOLOGY

Human health is undeniably a topic of intense research focus today. It's an issue that affects each one of us personally—including our friends, family, and colleagues—while also having a global impact. We are constantly bombarded by environmental insults that can affect our health, from the relatively innocuous irritants that cause allergies to potentially deadly viruses, parasites, and bacteria. Our most effective defense against these microscopic invaders is our immune system. However, this de-

fense mechanism can also turn against itself, causing a range of autoimmune diseases. Despite rapid progression in the field of immunology over the past few decades, scientists are still searching for a deeper understanding of the intricacies of the immune system and the relationships between host and pathogen. New discoveries hold the potential for uncovering novel strategies to strengthen our immunological defense mechanisms and prevent their malfunctioning.

One institution that has focused on unraveling some of the remaining questions in immunology is the RIKEN Research Center for Allergy and Immunology (RCAI). Since 2001, RCAI has published numerous research papers that have significantly advanced our knowledge in different areas of immunology. In this booklet, we invite you to read a sampling of RCAI publications that



have shed light on topics such as immune cell development and differentiation, the protective role of probiotics, immune system homeostasis, and the role of immune cells in cancer and autoimmune diseases.

Many challenges lie ahead for immunologists as the field becomes increasingly multidisciplinary and as advancing life-science technologies enable larger and larger data sets to be collected and analyzed. We are encouraged to see institutions such as RCAI look toward the future and bring together top-notch researchers from around the world to interpret important new findings in immunology, draw from one another's expertise, and drive immunology forward to find new ways of improving human health.

Tianna Hicklin, Ph.D. and Sean Sanders, Ph.D.
Editors, *Science/AAAS* Custom Publishing Office

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THE RIKEN RESEARCH CENTER FOR ALLERGY AND IMMUNOLOGY CELEBRATES ITS FIRST DECADE

The RIKEN Research Center for Allergy and Immunology (RCAI) has been one of Japan's premier strategic natural science research institutes since its establishment in 2001.

Two key principles have guided the philosophy and direction of RCAI research in its first decade: 1) *creating new paradigms in basic immunology* and 2) *bridging basic immunology findings and medical innovation*. RCAI scientists explore many areas of immunology, including molecular imaging, immune system development, cell signaling, genetic and epigenetic regulation, mucosal immunity, tumor immunity, and the development of new therapies and diagnostic tools. Since the center's inception, RCAI researchers have had a consistently strong track record, continuously publishing papers in distinguished scientific journals; more than 30 percent of our papers published yearly are found in journals with an impact factor greater than 10. Moreover, the number of citations per paper has been consistently above 40. RCAI's immunology research is highly ranked, with a high citation index score, and compares favorably with other premier immunology institutions around the world. This *Science* reprint collection booklet summarizes some of RCAI's major research findings over the last decade and introduces a selection of RCAI papers published in *Science*, *Science Signaling*, and *Science Translational Medicine*.



The immune system is critical for maintaining homeostasis in the body, is a key target for developing therapeutics, and is important for preventing a variety of diseases. RCAI scientists have made many discoveries in basic immunological research using in vitro and ex vivo techniques and animal models; moreover, our researchers have also seen significant progress with translational research studies, including the development of “humanized” mice, preclinical work on a cedar pollen allergy vaccine, and clinical trials investigating a natural killer T (NKT) cell therapy for cancer. In its second decade, RCAI has focused on developing ways to relate basic research findings to understanding the human immune system. In 2011, RCAI launched the Medical Immunology World Initiative (MIWI), a new human immunology consortium of laboratories that uses humanized mice. MIWI will serve as a worldwide network for the next generation of human immunology research by virtue of its focus on integrative medical immunology.

Building on the groundwork laid by RCAI, RIKEN has decided to establish a new research center for integrative medical science that will launch in April 2013. The research conducted during RCAI's first 11 years has opened the door to new directions in human immunology, and the current endeavors of RCAI investigators continue to spearhead global immunology research, connect scientists around the world, and improve human well-being.

Masaru Taniguchi, M.D., Ph.D.
Director
RIKEN Research Center for Allergy and Immunology

THE INTERCONNECTION, INTERDEPENDENCE, AND INFLUENCE OF THE IMMUNE SYSTEM

Immunology is a field of discoveries, inventions, and breakthroughs. From antibodies to vaccines, the products of immunology research have transformed basic science and clinical practice. Today, immunologists are addressing major medical and scientific challenges such as the global rise in inflammatory disease, autoimmunity, and allergy; the complex interaction between genes and environment; and the need for individualized and effective immunotherapies. Immunology researchers are using powerful, high throughput techniques and a variety of in vivo models to address these challenges. Immunology continues to be a dynamic field of research that provides tools and inspiration to clinical and basic research.

Immunology has a rich history of groundbreaking discoveries, from Edward Jenner's successful smallpox vaccinations in the 18th century to Susumu Tonegawa's Nobel Prize-winning explanation of antibody gene recombination 200 years later. Immunology research in the 21st century is discovering that the innate and adaptive immune systems are more intricately connected than previously thought. In fact, the entire immune system is now viewed as a complex communication hub, coordinating interactions between the external world of pathogens, commensal microorganisms, and allergens, and our internal physiology from organs to genes. This model of interdependency underlies current immunology research, whether the topic is early immune development, gene-environment interactions, receptors and signaling, or clinical therapies.

A great deal of current immunology research is driven by the global rise in rates of autoimmune disorders, allergy, and inflammation-related diseases. An explanation for this global pattern, says Harald Renz, professor of Laboratory Medicine, Department of Clinical Chemistry and Molecular Diagnostics, Philipps University, Marburg, Germany, is the hygiene hypothesis—that early exposure to a variety of antigens builds a stronger, better-functioning adult immune system. A hyperhygienic environment in early life inhibits the development of a proper balance between the actions of T effector and T regulatory cells. “We have an immune system to fight infections, and if you don't test the immune system with exposure to pathogens, you lose homeostasis, resulting in immune dysregulation,” says Joan M. Goverman, chair of the Department of Immunology, University of Washington, Seattle.

In spring 2012, experimental findings using an animal model provided support for the hygiene hypothesis. Richard Blumberg, chief of the Division of Gastroenterology, Hepatology and Endoscopy of Brigham and

Women's Hospital in Boston and colleagues found that germ-free mice are more susceptible to inflammatory conditions resembling colitis and asthma and traced this susceptibility to increased invariant natural killer T cells (NKT). Early exposure to the normal commensal microorganisms of mice resulted in normal NKT cell levels, leading the researchers to conclude that “age-sensitive contact with commensal microbes” established appropriate microbial tolerance. Normalization of the immune system occurred only when microbial exposure was early in life, and not if it occurred in adulthood.

Investigating Earlier and Broader Influences

In fact, research on the early immune system is finding that effects that are prenatal or even earlier can influence immune system development. For example, mouse models show that maternal and even grandmaternal exposures to nutrients, pollutants such as cigarette smoke, and infectious agents such as bacteria can influence immune development. These transgenerational influences are explained by epigenetic effects, which are heritable changes in the accessibility of DNA to transcription factors through modifications to nucleotides or histone proteins. For instance in mice, certain concentrations of the methyl donor folate in the maternal diet resulted in epigenetic effects to genes linked to allergic airway disease. This effect was transmitted to a second generation. Renz's group found that prenatal exposure to the soil bacterium *Acinetobacter lwoffii* protected against asthma in a mouse model, and was associated with epigenetic effects on cytokine genes such as interferon-gamma and interleukin-4. The next steps in immunology research are identifying the specific agents, and the timing and type of exposure that give protective effects against later immune dysregulation. Researchers are also looking for the targets of early environmental exposure,

such as stem and progenitor immune system cells. “This is a field to watch,” says Rafeul Alam, head of allergy and immunology at National Jewish Health and professor of medicine at University of Colorado Denver. Says Alam, “Work on epigenetics so far has just scratched the surface. This is fertile ground for research.”

Immunology researchers are also broadening their definition of the environment. They are finding that factors affecting immune development include “food and dietary habits, obesity and metabolic syndrome, psychological factors such as stress, and neuroendocrine interactions,” says Renz. “A big area is microbial exposure, including microbes that normally colonize the mucosa of the respiratory system, gut, and skin. These microbes have a strong impact early in life beyond the risk of allergy to risk of conditions such as type I diabetes, multiple sclerosis, neurodegenerative disease, schizophrenia, and depression.”

Research on commensal gut microbes has found that colonization of the gastrointestinal tract, mainly by species in the phyla *Firmicutes* and *Bacteroidetes*, begins immediately after birth. In mice, intestinal colonization promotes development of immune responses including production of antimicrobial peptides, differentiation of helper T cells, development of T regulatory cells, and secretion of IgA. This is a symbiotic relationship that regulates growth of commensal bacteria that aid in digestion and produce vitamins and other nutrients for the host. Crosstalk clearly occurs between the microbiota and the immune system, and current research is investigating both sides of this communication. Active research areas include studying how immune development and maintenance are affected by specific species and strains of microbes, particular compositions of the microbiome, and the amount of microbiome diversity. In humans, the main method for this type of research is sampling and characterizing the human microbiome, for example by high throughput pyrosequencing in the U.S. National Institutes of Health Human Microbiome Project. Analysis is underway to discover associations between the presence of specific microbes, longitudinal changes in the human microbiome, and conditions ranging from obesity to autoimmune disease. This will determine which microbes are our friends and which are our foes—the basic task of the immune system.

Self and Nonself

Distinguishing between pathogens and commensals and self and nonself is a fundamental function of the immune system. When this functionality goes awry, the result can be autoimmunity. Even a correctly functioning immune system can cause transplant rejection or graft vs. host disease. Substantial research with the goal of modulating the immune system has elucidated many of the mechanisms by which antibodies, T cell receptors, major histocompatibility complexes, and other immune system elements determine which cells to attack and which to tolerate. Recent attention has focused on pattern recognition receptors, particularly the toll-like receptors (TLRs). These transmembrane and intracellular proteins, with the help of adaptor proteins, bind to foreign molecules such as bacterial lipopolysaccharides or viral RNAs and signal pathogen invasion. Discovery of the immune functions of TLRs was awarded the 2011 Nobel Prize for Physiology or Medicine.

Activation of TLRs by ligand binding initiates kinase-dependent signal transduction cascades that lead to gene expression responses mediated by transcription factors such as NF κ B. Professor Alam says that a current area of research is investigating just how the TLR pathways lead to the activation of inflammation, a topic that is particularly urgent given the global health burden of chronic inflammatory conditions such as diabetes and inflammatory bowel disease. Alam says we need additional research on how pathogen signals reach and trigger intracellular TLRs such as TLR7 and TLR9. Although we have a lot to learn from TLRs, he says, future research should also focus on other receptors connected to inflammation such as tyrosine kinase-associated receptors and other RNA- and DNA-sensing receptors. Nucleic acid-sensing receptors are particularly important in understanding autoimmune disease since many of these are an inappropriate reaction to RNA or DNA.

Much research on the response to receptor signaling has focused on changes in gene transcription, but Alam advises more attention to downstream gene expression effects such as alternative splicing, microRNA regulation, and protein modification. “It’s simple logic,” he says. “Higher mammals have fewer genes in their genomes than other organisms, so posttranscriptional and post-translational modifications have a bigger impact in creating different phenotypes.”

Directing the Immune System for Therapy

As our understanding of the internal and external communications and responses of the immune system grows, the list of potential therapeutic targets and technologies expands. Development of monoclonal antibodies, for which César Milstein, Niels Kaj Jerne, and Georges J. F. Köhler shared the 1984 Nobel Prize in Physiology or Medicine revolutionized basic research by providing tools for precisely identifying, isolating, or marking cellular components. Monoclonal antibody technology has led to highly specific therapies such as the breast cancer treatment trastuzumab (Herceptin). The success of Herceptin and similar therapies has launched dozens of biotechnology companies and clinical trials. Gorman, who studies autoimmune diseases such as multiple sclerosis, says, “Cancer therapy is driving the development of therapy for autoimmune diseases and other immune disorders.” For example, a monoclonal antibody therapy used to treat malignant B-cell lymphomas has shown some promise with multiple sclerosis and diabetes. Learning about its mechanism of action has also informed multiple sclerosis research.

On the cell-based side, in 2010, the Seattle company Dendreon received U.S. Food and Drug Administration approval for their prostate cancer treatment, sipuleucel-T (Provenge). The therapy stimulates the patient’s own peripheral blood mononuclear cells to launch a specific attack against cancerous cells. Commercialization of Provenge remains rocky, but the clinical proof-of-concept has motivated additional cell-based therapy development. Poul Sørensen, senior vice president of strategic development, Stallergenes, Paris, and board member of the Danish Graduate School of Immunology, says these therapies are promising, but the double-edged sword in any type of cell-based immunotherapy is the inherent plasticity of immune cells. We are learning how easily immune cells change function, he says, for example converting from anti-inflammatory to proinflammatory. This cellular flexibility is a challenge in large-scale cell culturing, the method which Dendreon must use in their Provenge therapy. However, immune cell plasticity also explains immunotherapy successes, for example treatment for specific allergies. Says Sørensen, “We’re learning we can reprogram the immune system with lasting effects. This has the potential to lead to treatment breakthroughs for other diseases.”

Cancer immunotherapy is “coming of age,” says Joseph Murphy, director of Cancer Therapeutics and Immunology, Southern Research Institute, Birmingham, Alabama, citing more than 40 different immunotherapies currently in testing in over 60 clinical trials. Murphy, who wrote an extensive 2010 review of cancer immunotherapy, says that current cancer immunotherapy is based on research showing that cancerous cells create an immune microenvironment that suppresses antitumor activity. He says that new cancer therapies will be “based on a sophisticated knowledge of immune-suppressive cells, soluble factors, and signaling pathways designed to break tolerance and reactivate antitumor immunity to induce potent, long-lasting responses.” Examples are highly specific, cell-free vaccines and other treatments based on tumor-associated antigenic peptides identified from a patient’s resected tumor. An integrated, multipronged immune response is required to eliminate cancer, so combinatorial approaches are likely to be the most effective. Future therapies, Murphy says, “will simultaneously address the immunosuppressive, angiogenic, invasive, and hypoxic nature of cancer.”

Other therapies are also developing in multiple directions. Rachna Shah, an allergist with Gottlieb Memorial Hospital, Loyola University Health System in Maywood, Illinois notes that studies on complementary and alternative therapies such as traditional Chinese medicine to treat allergies are becoming more common. “Both patients and providers are interested in seeing if these treatments can make a difference in symptoms,” she says. In conventional therapy, says Shah, oral immunotherapy is a major trend, with recent trials showing promise in sublingual therapy for egg, peanut, and pollen allergy.

Research on the pathogenesis of infectious diseases such as influenza and SARS could contribute to the development of immunomodulating therapies. Influenza and other viral infections can induce a cytokine storm in which strong production of complement factors and proinflammatory cytokines brings excessive numbers of inflammatory cells to the lungs, ultimately impairing lung function. Learning to modulate immune system communications in this situation will have clinical implications beyond infectious disease.

Looking Ahead

Future immunology research will continue to explore the extraordinary complexity of the immune system and its influence on health and homeostasis. Driving current research are recent discoveries about the intricate functions of the innate immune system and its interconnection with the adaptive system. Goverman says we are now realizing that innate immune system cells such as dendritic cells and macrophages not only acquire and present antigens to T cells, but do so in varied contexts that convey different instructions to the T cells, depending on the pathogen. Elucidating the molecular mechanisms that define these conversations between innate immune cells and T cells—the receptors on dendritic cells that encounter different pathogens, the signaling pathways they activate, and the cell-surface molecules expressed to orchestrate the adaptive immune response—is a research frontier that immunologists will continue to explore. Shah agrees. Immunologists know that the innate and adaptive systems interact, she says, but now are “discovering the level of interdependence between the different parts of the immune system and how these connections affect disease.” Another research frontier, says Goverman, is investigating different cell-death pathways, because “the way a cell dies can initiate different immune responses.”

A major breakthrough in in vivo research has been the development of humanized mice, says RIKEN Research Center for Allergy and Immunology (RCAI) Director Masaru Taniguchi. Increasing numbers and types of human genes and cells are being introduced into mice for studying human disease and normal human immune development. Fumihiko Ishikawa, group director of the Lab for Human Disease Models, RIKEN RCI, is a leader in the creation of humanized mice. As with other areas of immunology, a major challenge is creating the proper complex environment for immune cell development—in this case the most human environment possible within mice. Ishikawa says, “We can produce human immune cells that differentiated from human hematopoietic stem cells in mice, but they develop in a mouse environment. A major future research direction will be better recapitulating the human environment in a mouse model.” Although only a model for human immunity, humanized mice are valuable for studying cancer—especially leukemia—and viral diseases, because many viruses such as HIV are species specific.

Microscale and nanoscale technologies such as single-cell imaging and single-molecule polymerase chain reaction (PCR) bring power and sensitivity to studies on receptor-ligand interaction and microRNA function. Second-generation sequencing of DNA and cDNA (complementary to RNA) is allowing systems biologists to move beyond microarray studies to more sophisticated analyses of genomic events. Using genome-wide association studies (GWAS), immunologists have discovered associations between particular polymorphisms or gene expression patterns and immune disorders. Goverman says that researchers are now taking the next step: exploring the underlying reasons and mechanisms for these associations.

Alam says that systems analysis can identify genes and other factors associated with immune conditions, but he emphasizes that we must always keep the complexity of the immune system in mind. For example, he says, GWAS identified specific genes such as those for interleukin-33 and its receptors as associated with susceptibility to asthma. However, this discovery has led to extensive research that is focused on only a few genes. Immunologists must realize they are studying an extremely complicated system that is linked to genetics, physiology, and the environment, says Alam. “Remember that even the best of the genes identified by GWAS explain at most 10 to 20 percent of a phenotype. There are so many other factors—other genes and especially environmental factors. Sometimes scientists like to be as reductionist as possible, to keep things clear, but real life is very complex.”

—Chris Tachibana, Freelance Science Writer



HIGHLIGHTS OF RCAI RESEARCH AND PROGRAMS: 2001–2012

Since its establishment in 2001, the Research Center for Allergy and Immunology (RCAI) has been at the cutting edge of immunology research and has focused on two goals: *creating new paradigms in basic immunology* and *developing medical innovations*. During the 11-year directorship of Masaru Taniguchi, RCAI researchers have made a big impact on understanding how the immune system works. Although basic research has always been a focus and strength of RCAI, the center has also developed programs with a translational component. Here, a sampling of the pioneering studies and programs that have contributed to RCAI's mission are briefly described.

Creating New Paradigms

1) Immune System Development

As the immune system develops, the progeny of multipotent hematopoietic stem cells (HSCs) become increasingly restricted in their lineage potential and acquire specialized functions. Scientists have long believed that lymphoid lineage precursors lack the ability to mature into myeloid lineage cells; however, Hiroshi Kawamoto's research showing that early thymic progenitors can give rise to myeloid cells has called this dogma into question (7). His group later discovered that the transcription factor Bcl11b determines the T cell lineage (see full paper on page 15) (2).

Ichiro Taniuchi's group provided further insight into what drives T cell differentiation. They found that the Runx complex represses the expression of the transcription factor Th-POK during thymocyte differentiation, allowing the development of CD8⁺ cytotoxic T cells rather than CD4⁺ helper cells (see full paper on page 19) (3, 4).

2) Immune Cell Signaling

Immune cells use receptor-mediated signaling at multiple stages in their development/differentiation to direct lineage choices and acquisition of effector functions. Changes in expression levels or posttranslational modification—such as phosphorylation—of signaling molecules propagate signals within immune cells. Tomohiro Kurosaki's group has dissected the molecular events in B cell signaling. They identified Stim1/Stim2 as critical for calcium (Ca²⁺) influx, a key event in many signaling pathways (5, 6), and the Ser/Thr kinase extracellular signal-regulated kinase (ERK) as essential for early B cell development and plasma cell differentiation (see abstract on page 23) (7, 8).

A surprising discovery was made by Toshio Hirano and his RCAI team, when they found that not only Ca²⁺, but also zinc (Zn²⁺) ions can act as signaling molecules.

They determined that Zn²⁺ signaling plays a role in cell migration, dendritic cell activation, allergic reactions, and connective tissue formation (9–12). Further, they identified two types of Zn²⁺ signaling: early, which involves a “Zn²⁺ wave,” similar to that observed during Ca²⁺ influx, and late, which is induced by the expression of Zn²⁺ transporters (13).

Visualization of signaling events at the single-molecule level is now possible using highly sensitive fluorescence microscopes developed by RCAI's Makio Tokunaga (14). Takashi Saito observed the dynamic movement of signaling molecules during T cell activation and discovered that activation is initiated by small complexes called “T cell receptor (TCR) microclusters,” in which TCRs and various signaling proteins are assembled (15, 16). The group could also visualize T cell activation modulated by costimulatory molecules. These findings have provided key insights into how the spatiotemporal patterns of signaling events affect the initiation, maintenance, and regulation of T cell activation (17, 18).

3) Mucosal Immunity

The adaptive immune system has two separate but interrelated components: systemic and mucosal. Much of classical immunology focuses on the systemic compartment; however, protection at mucosal surfaces is also essential for survival. The “lymph-node equivalent” in the intestine are Peyer's patches (PPs), which are separated from the intestinal contents by a single layer of specialized epithelial cells, among which are antigen-sampling M cells that deliver antigen to PPs to initiate mucosal immune responses, but the mechanisms by which M cells differentiate and carry out this function have not been fully elucidated.

Hiroshi Ohno and his colleagues discovered that a transcription factor, Spi-B, is essential for M-cell differ-

entiation (19) and that glycoprotein-2 (GP-2) serves as a bacterial uptake receptor (20). The intestinal immune system has intimate and important interactions with the bacterial flora in the gut. Using integrated multi-“omics” approaches, Ohno’s team studied the “probiotic” effect of these interactions. They discovered that in a mouse model, bacteria from the genus *Bifidobacterium* produced acetate that prevented Shiga toxin generated by enterohemorrhagic *E. coli* O157 from entering the bloodstream, thereby protecting the mice (21).

Moreover, a group led by Sidonia Fagarasan investigated the role of immunoglobulin A (IgA) in maintaining the symbiotic balance between gut microbiota and the host immune system (22). In collaboration with Shohei Hori, they demonstrated that environmental cues in the gut promote the selective differentiation of IgA-supporting T follicular B helper (T_{FH} cells) from $Foxp3^+$ T cells (see abstract on page 25) (23). Fagarasan’s group also recently found that skewing of the microbiota, which results from IgA with reduced bacteria-binding capacity, drives the generalized activation of B and T cells in mice that develop B cell-dependent autoimmune disease (see full paper on page 11) (24).

4) Systems Immunology

RCAI is actively creating new immunology fields that are integrated with other scientific fields as well as new technologies. One example is systems immunology, which fuses biology and mathematics.

RCAI’s Mariko Okada, in collaboration with Boris Kholodenko (University College Dublin), has used mathematical modeling to tackle the question of how cell fate is determined. Epidermal Growth Factor (EGF) and Heregulin (HRG) both share extracellular signal-regulated kinase (ERK) signaling pathways, but induce distinct cell fates; EGF induces cell division, while HRG induces differentiation. The group found that EGF and HRG induce either transient or sustained cytoplasmic ERK activity, respectively, and that discrimination of these signals controls cell fate (25).

New imaging technologies have the potential to become important tools in systems biology since the information obtained can be digitized and quantified. A step in this direction was taken by Takaharu Okada, who utilized two-photon microscopy to track B cells by moni-

toring the expression of Bcl6 with a surrogate fluorescent marker. His team could observe, track, and quantify the in vivo migration of Bcl6-expressing antigen-specific B cells to the germinal center, a process dependent upon interaction with Bcl6-expressing T_{FH} cells (26).

—Takashi Saito, Deputy Director, RIKEN RCAI

Developing Innovations in Biomedical Sciences

1) Humanized Mice

RCAI scientists are working to develop innovative experimental models for investigating the human immune system and disease. RCAI researchers Fumihiko Ishikawa, Haruhiko Koseki, and Osamu Ohara, in collaboration with Leonard Shultz (Jackson Laboratory), have generated immunodeficient mice (NOD/SCID/IL-2rgKO (NSG)) that express a human class I antigen. When newborn mice were transplanted with human hematopoietic stem cells (HSCs), human cytotoxic T cells successfully developed and became functional in these “humanized” mice (27). More recently, this group created an improved recipient mouse (hSCF Tg NSG) that expresses human stem cell factor (SCF), a cytokine that is critical for the maintenance of stem and progenitor cell activities (28). Ishikawa also identified a human leukemia stem cell (LSC) by transplanting human acute myeloid leukemia (AML) cells into NSG mice. The LSCs propagate the leukemia, but are typically in a dormant state and therefore resistant to cell cycle-dependent chemotherapy (29–31). His group is currently identifying genes differentially expressed between normal HSCs and LSCs to identify potentially new therapeutic targets for AML (see abstract on page 24).

2) Natural Killer T Cell Therapy

A clinical trial of a natural killer T (NKT) cell targeted therapy for patients with advanced non-small-cell lung cancer is being conducted by RCAI’s Masaru Taniguchi, in collaboration with Chiba University. In this trial, autologous dendritic cells pulsed with the NKT cell-activating ligand α -galactosylceramide were administered intravenously. Sixty percent of treated patients (10/17) showed a significantly longer median survival compared to the

HIGHLIGHTS OF RCAI RESEARCH AND PROGRAMS: 2001–2012

untreated group (29.3 versus 4.6 months). Based on these promising results, expanded trials of the NKT cell therapy are planned, and this treatment is now offered as a new therapeutic option under the advanced medical care assessment system approved by the Japanese Ministry of Health, Labor and Welfare. Thus, this treatment will be covered in part by national health insurance (32).

3) Primary Immunodeficiency Clinical Archive

To promote awareness and improve treatment options for Primary Immunodeficiency (PID), RCAI has been expanding its collaborative network with the clinical study group from 13 Japanese universities, the Institute of Bioinformatics in India, and the Kazusa DNA Research Institute, supported in part by the Jeffrey Modell Foundation. The number of patient samples deposited in the Japanese clinical archive, “PIDJ,” which was established by RCAI, continues to increase each year. RCAI is also working toward establishing an Asian PID network and has established an integrated bioinformatics platform for PID called RAPID (rapid.rcai.riken.jp) and a PID mutation annotation server, Mutation@A Glance (rapid.rcai.riken.jp/mutation).

—Toshitada Takemori, Research Coordinator, RIKEN RCAI

Toward the Future

1) Medical Immunology World Initiative (MIWI)

To understand the process of disease development and to identify critical events that change human health, RCAI established an interdisciplinary biomedical research platform and international consortium of research groups that share a common interest in human immunology. For this purpose, RCAI launched the zMedical Immunology World Initiative (MIWI) as a new human immunology platform that includes humanized mice. Nine institutions are currently affiliated with MIWI: the *Immunology Frontier Research Center of Osaka University* (IFReC), the *U.S. National Institutes of Health* (NIH), the *Institute of Medical Science*, the *University of Tokyo* (IMSUT), two departments from *Zurich University*, *INSERM/Necker Hospital*, the *Pasteur Institute*, *Imperial College London*, and RCAI. The goal of

MIWI is to use integrative immunological approaches to obtain fundamental knowledge about the human immune system and the underlying mechanisms of disease development and to discover new principles for diagnosis and treatment.

2) Young Chief Investigator Program (YCI)

RCAI launched the new Young Chief Investigator (YCI) Program to provide a career path for young investigators (under 40 years old) conducting multidisciplinary research that bridges immunology with other fields. A YCI will head an independent laboratory but will have access to mentors who are specialists in related fields. Five researchers have been selected thus far to conduct pioneering work in the following new areas: stem cells and aging reversal, development of the interface between integrative biology and mathematics, mucosal flora and epigenetic analysis, immune regeneration, and multi system-wide analysis.

—Shigeo Koyasu, Deputy Director, RIKEN RCAI

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The Inhibitory Receptor PD-1 Regulates IgA Selection and Bacterial Composition in the Gut

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Immunoglobulin A (IgA) is essential to maintain the symbiotic balance between gut bacterial communities and the host immune system. Here we provide evidence that the inhibitory co-receptor programmed cell death-1 (PD-1) regulates the gut microbiota through appropriate selection of IgA plasma cell repertoires. PD-1 deficiency generates an excess number of T follicular helper (T_{FH}) cells with altered phenotypes, which results in dysregulated selection of IgA precursor cells in the germinal center of Peyer's patches. Consequently, the IgAs produced in PD-1-deficient mice have reduced bacteria-binding capacity, which causes alterations of microbial communities in the gut. Thus, PD-1 plays a critical role in regulation of antibody diversification required for the maintenance of intact mucosal barrier.

The primary function of immunoglobulin A (IgA) is to maintain homeostasis at mucosal surfaces. Intestinal IgA production occurs via both T helper cell-dependent and independent pathways (1). The diversification of IgA repertoire by somatic hypermutation (SHM), however, takes place mostly in specialized microenvironments called germinal centers (GCs), in which B cell interaction with T follicular helper (T_{FH}) cells induces the expression of activation-induced cytidine deaminase (AID) (2, 3). T_{FH} cells express high amounts of the inhibitory co-receptor programmed cell death-1 (PD-1) (4). PD-1 deficiency leads to species-specific, antibody-mediated autoimmune diseases (5–7). Note that the incidence of diseases in PD-1-deficient mice varies among mouse colonies and depends on AID (8).

We investigated whether PD-1 regulates microbial communities and IgA production in the gut. Although the total number of bacteria cultured from the lumen of the small intestine was comparable between PD-1-deficient mice (*Pdcd1*^{−/−}) and wild-type (WT) mice (4.8×10^8 and 4.7×10^8 bacteria per g of intestinal content, respectively), *Pdcd1*^{−/−} mice had a 93 to 95% reduction in the number of anaerobic bacteria compared with WT mice (average 2.86×10^8 and 0.18×10^8 in WT and *Pdcd1*^{−/−} mice, respectively) (Fig. 1A). The total numbers of “healthy” bacteria, such as *Bifidobacterium* and *Bacte-*

roides (9) were not detectable or markedly reduced in *Pdcd1*^{−/−} mice. In contrast, bacteria of the *Enterobacteriaceae* family, which were minor representatives in the WT mice, were increased about 400-fold in *Pdcd1*^{−/−} mice. These results were confirmed by 16S ribosomal RNA (rRNA) gene pyrosequencing of cecal contents (fig. S1A). Thus, PD-1 deficiency perturbs the balance of bacterial communities in the gut.

The frequencies and the absolute numbers of IgA-producing cells in the lamina propria (LP) were comparable in *Pdcd1*^{−/−} and WT mice (fig. S1, B, C, and F). Flow cytometric analyses of fecal bacteria revealed, however, that the proportion of bacteria coated with IgA (bound IgAs) was reduced in *Pdcd1*^{−/−} mice (10) (Fig. 1B). In contrast, the concentration of free IgA in intestinal secretions was higher in *Pdcd1*^{−/−} than in WT mice (Fig. 1C). To assess the features of LP IgA, we sequenced the immunoglobulin heavy chain (IgH) genes in single sorted IgA-producing cells. Both WT and *Pdcd1*^{−/−} mice had a diverse IgA repertoire, yet *Pdcd1*^{−/−} mice had an enrichment of IgA-producing cells with the IgH locus belonging to non-V_{H1} family genes (Fig. 1D). This difference in the IgA repertoire may be the result of the altered composition of the microflora in *Pdcd1*^{−/−} mice. Indeed, as signs of antigen-mediated selection in their IgH genes, about 90% of the sequences from WT and *Pdcd1*^{−/−} mice had mutations and high ratios of replacement (R) to silent (S) mutations in complementarity-determining region 1 (CDR1) and CDR2, compared with those in framework regions 1 to 3 (FWR1-3) (fig. S1, D and E). However, the calculated affinity maturation index (11, 12) was lower in IgA-producing cells from LP of *Pdcd1*^{−/−} mice (Fig. 1E). Together, the results indicate that alterations of IgA compartment in *Pdcd1*^{−/−} mice have an impact on symbiotic relations between host and commensal bacteria in the gut.

The altered repertoire of the IgA plasma cells in gut could result from changes in the

turnover of IgAs at their inductive or residential places. Most of the IgA⁺ B cells are generated in the GCs of Peyer's patches (PPs) and arrive at the LP as plasmablasts [B220⁺ IgA⁺ MHCII⁺ (major histocompatibility class II-positive), hereafter, PBs] (13). In the LP, PBs down-regulate the B cell receptor and MHCII and differentiate into plasma cells (B220[−] IgA⁺ MHCII^{low} CD138⁺, hereafter, PCs) (fig. S1F). The proliferation of IgA-producing cells in LP was assessed using fluorescent ubiquitination-based cell-cycle indicator (Fucci) mice, in which the cells in S-G₂-M phase of the cell cycle are fluorescently marked (14). Similar to humans, most of the proliferating cells were PBs (13, 15), and the frequency of PBs in the cell cycle was comparable for *Pdcd1*^{−/−} mice and WT mice (fig. S1F).

We next determined the turnover of IgA-producing cells in LP based on 5-bromo-2'-deoxyuridine (BrdU) incorporation. Mice were continuously fed BrdU for 10 days, and then BrdU was removed from their drinking water. Ten days after the initiation of BrdU administration, there were significantly more BrdU⁺ PBs in the LP of *Pdcd1*^{−/−} mice than in those of WT mice (Fig. 1F). Five days after the removal of BrdU, however, the frequency of BrdU⁺ PBs was reduced by half in *Pdcd1*^{−/−} mice, whereas the frequency of BrdU⁺ PBs in WT mice remained unchanged (Fig. 1F). In contrast, the frequency of BrdU-labeled PCs did not differ significantly between *Pdcd1*^{−/−} and WT mice (Fig. 1F). The higher turnover of *Pdcd1*^{−/−} PBs may be the result of increased apoptosis. Cell death was assessed by detection of caspase activation. Compared with WT mice, we observed a significant increase in frequency of caspase^{hi} PBs and PCs in the LP of *Pdcd1*^{−/−} mice, but this diminished after antibiotic treatment (Fig. 1G and fig. S1G). Together, these results indicate enhanced commensal-driven turnover of IgA-producing cells in LP of *Pdcd1*^{−/−} mice.

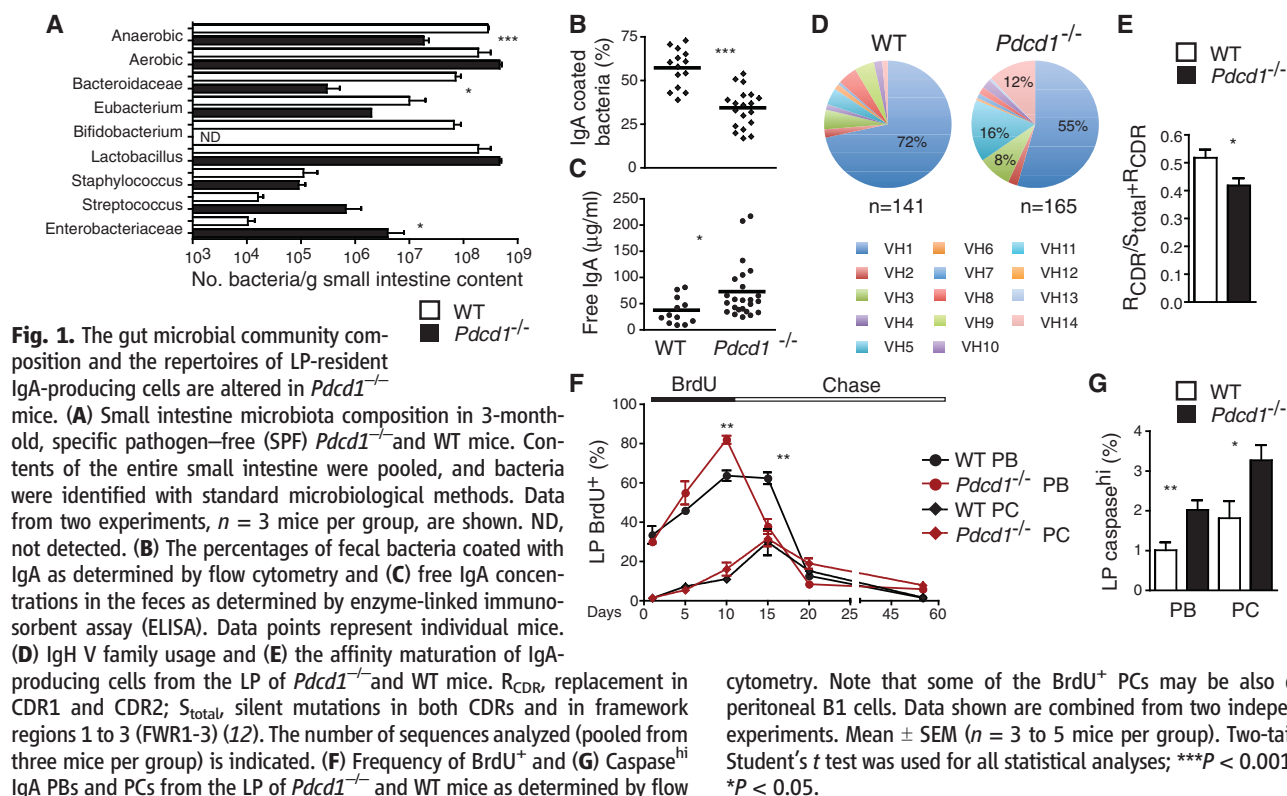
Next, we examined the PPs of *Pdcd1*^{−/−} mice. Compared with WT mice, *Pdcd1*^{−/−} mice had significantly more peanut agglutinin-positive (PNA⁺) Fas⁺ GC B cells and IgA⁺ B cells in PPs (Fig. 2, A to C). Although we observed a modest increase in the frequency of light-zone B cells in the PP GCs of *Pdcd1*^{−/−} mice (16) (fig. S2A), quantitative real-time fluorescence polymerase chain reaction analyses of several important B cell markers for dark-zone and light-zone GC B cells did not show significant differences between *Pdcd1*^{−/−} mice and WT mice (fig. S2B). A comparable proportion of IgA⁺ GC cells from *Pdcd1*^{−/−} and WT mice were in cell cycle, as assessed by Fucci or a 12-hour BrdU pulse test (17) (fig. S2, A and C). The pulse-chase BrdU experiment revealed considerable differences in dynamics of IgA GCs in *Pdcd1*^{−/−} mice, however. Namely, in both *Pdcd1*^{−/−} and WT mice, the frequency of GC and IgA⁺ B cells that incorporated BrdU reached a plateau of 95 and 80%, respectively, 5 days after the initiation of BrdU labeling (Fig. 2D). However, 5 days after the

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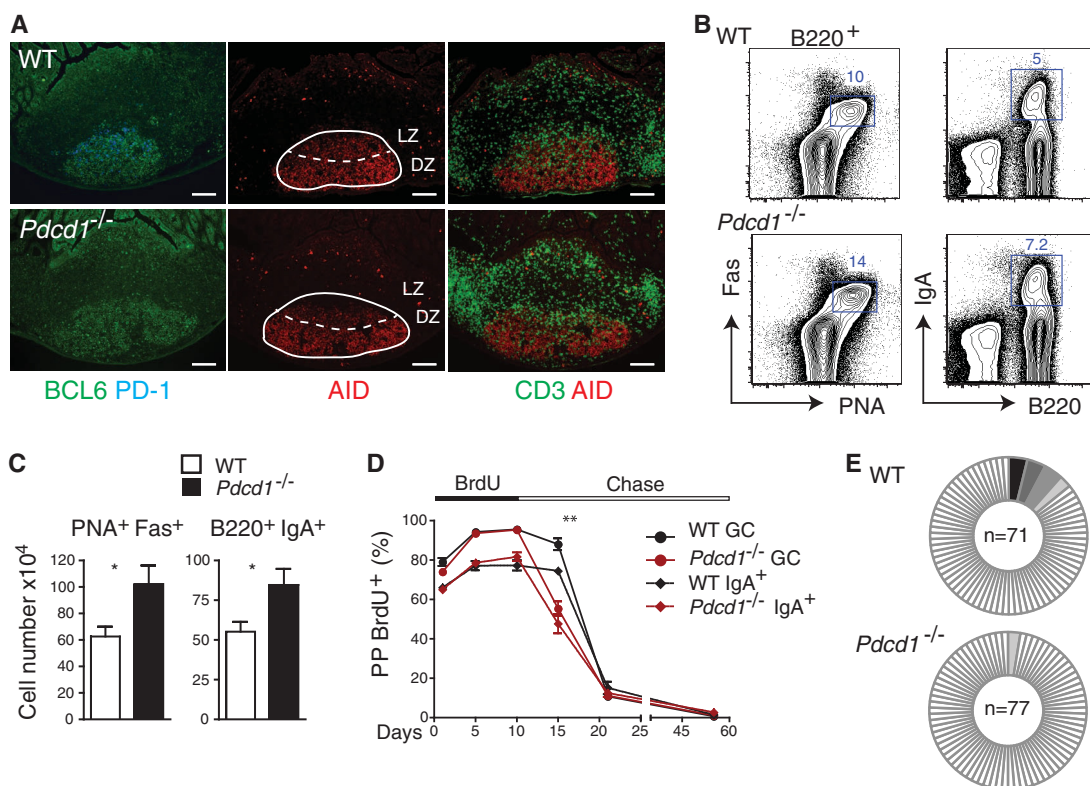
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cytometry. Note that some of the BrdU⁺ PCs may be also derived from peritoneal B1 cells. Data shown are combined from two independent sets of experiments. Mean $\pm$ SEM (*n* = 3 to 5 mice per group). Two-tailed unpaired Student's *t* test was used for all statistical analyses; ****P* < 0.001; ***P* < 0.01, **P* < 0.05.



cells from PPs of *Pdc1*^{-/-} and WT mice as determined by flow cytometry. Data are combined from two independent sets of experiments. Means $\pm$ SEM (*n* = 3 to 5 mice per group). **(E)** Representative charts of clonal diversity, calculated as frequency of transcripts of a given in-frame V_H-(N)-D-(N)-J_H rearrangement of GC IgA⁺ B cells in PPs of *Pdc1*^{-/-} and WT mice. The number of productive sequences compared is indicated. Two-tailed unpaired Student's *t* test was used for all statistical analyses; ***P* < 0.01; **P* < 0.05.

removal of BrdU, the frequency of labeled GC and IgA⁺ B cells was reduced by half in *Pdcd1*^{-/-} mice, whereas most of the BrdU⁺ cells remained in the PPs in WT mice (Fig. 2D). The significant drop in BrdU⁺ cells in PPs of *Pdcd1*^{-/-} mice could be the result of increased cell death in situ. However, there were no differences in the frequency of caspase^{hi} GCs and IgA⁺ cells for WT and *Pdcd1*^{-/-} mice (fig. S2D). The data suggest that the increased turnover of IgA⁺ B cells in PPs of *Pdcd1*^{-/-} mice may be because they pass through the GCs more rapidly. Also, the increased number of GC B cells in *Pdcd1*^{-/-} mice likely results from more inflow of B cells into GCs. Indeed, the frequency and numbers of IgD⁺ cells with an early GC phenotype (B220⁺IgD⁺IgA⁺GL7⁺Fas⁺CCR6⁺) (18) were higher in PP of *Pdcd1*^{-/-} mice (fig. S2, E and F). Taken together, our results suggest that PD-1 deficiency is associated with an increased import and export of B cells into the GC. Such an abbreviated transit time likely results in impaired clonal selection and expansion of IgA⁺ B cells in GCs of PPs, as shown by the reduction

of the frequency of clonally related IgA sequences [identical V_H(N)-D(N)-J_H where V_H is the variable region of the immunoglobulin heavy chain, N is a random nucleotide, D is the diversity region, and J_H is the joining region of the immunoglobulin heavy chain] in *Pdcd1*^{-/-} mice compared with WT mice (Fig. 2E).

We then asked why PD-1 deficiency causes such changes in the dynamics of GC B cells in PPs. It is well established that the number and activation status of T cells—and T_{FH} cells in particular—play a fundamental role in shaping GC biology. In fact, B cells compete for T cell help before entry into GCs, as well as within GCs, and deregulation of T_{FH} cells leads to inappropriate GC B cell selection and humoral autoimmunity (16–19). Thus, we assessed T_{FH} cells in the PPs of *Pdcd1*^{-/-} mice. The frequency and absolute number of CD4⁺ T cells, including CXCR5^{hi}ICOS^{hi} (high levels of ICOS, protein-inducible costimulator) T_{FH} cells, were significantly increased in PPs of *Pdcd1*^{-/-} mice (Fig. 3, A and B, and Fig. 2A). The ratio of T_{FH} to GC B cells in *Pdcd1*^{-/-} mice was twice as high as

that in WT mice (Fig. 3C). The expression of BCL6, a transcription factor required for T_{FH} differentiation, was higher in all CD4⁺ T cell subsets, including the T_{FH} cells in PPs of *Pdcd1*^{-/-} mice (Fig. 3A). In contrast, the expression of interferon regulatory factor 4 (IRF4), which is required for the production of interleukin-21 (IL-21), the cytokine that promotes growth and differentiation of IgA⁺ B cells into PCs (19, 20), was reduced in T_{FH} as well as CXCR5^{hi}ICOS^{int} cells from *Pdcd1*^{-/-} mice. Indeed, T_{FH} cells from *Pdcd1*^{-/-} mice produced less IL-21 compared with those from WT mice, as previously reported (21) (Fig. 3, D and E). Furthermore, the proportion of T_{FH} cells producing interferon-γ (IFN-γ) was increased in PPs of *Pdcd1*^{-/-} mice.

In order to better characterize how T_{FH} cells may contribute to the altered GC responses observed in *Pdcd1*^{-/-} mice, we decided to evaluate the ability of PD-1-deficient T_{FH} cells to support IgA generation in gut in vivo. Thus, PP CXCR5^{hi}ICOS^{hi} T_{FH} cells were isolated from *Pdcd1*^{-/-} and WT mice and were adoptively transferred into T cell-deficient (lacking the

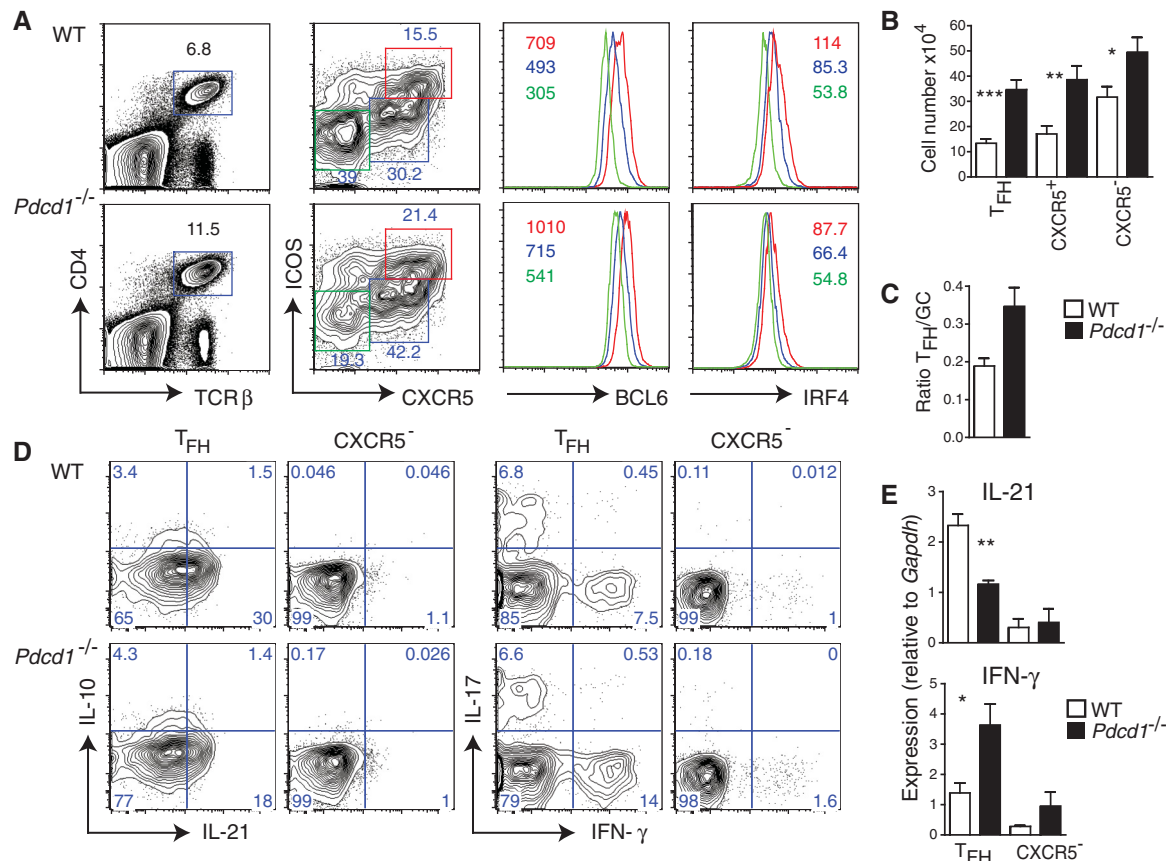


Fig. 3. Expansion of activated T cells and skewed cytokine profiles of T_{FH} cells in PP GCs of *Pdcd1*^{-/-} mice. (A) Representative flow cytometric profiles and plots of PP cells from WT and *Pdcd1*^{-/-} mice stained for the indicated markers. Numbers in the graphs indicate the geometric mean fluorescence intensity of BCL6 and IRF4 in the corresponding color-coded T cell subset gates. (B) Absolute numbers of major T cell populations and (C) the ratio of T_{FH} cells to GC B cells in the PPs of *Pdcd1*^{-/-} and WT mice. Means ± SEM (*n* =

at least 5 mice per group). (D) Flow cytometric profiles and (E) mRNA expression of indicated cytokines in PP T cell subsets. Numbers represent percentage of cells in the quadrants or gates. Relative amounts of mRNA normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) are shown. Means ± SEM of at least two independent experiments. Two-tailed unpaired Student's *t* test was used for all statistical analyses; ****P* < 0.001; ***P* < 0.01, **P* < 0.05.

CD3 ϵ subunit, *Cd3e*^{-/-} mice. Injection of T_{FH} cells from both WT and *Pdcd1*^{-/-} mice into *Cd3e*^{-/-} mice reconstituted the GC B cells and B220⁺IgA⁺ B cells in PPs (Fig. 4, A and B). However, *Cd3e*^{-/-} mice transferred with PD-1-deficient T_{FH} cells generated very few IgA-secreting cells in the LP compared with mice injected with WT T_{FH} cells (Fig. 4, A to C). The few IgAs induced by *Pdcd1*^{-/-} T_{FH} cells had increased turnover (which mirrored the intact *Pdcd1*^{-/-} mice) and a comparable number of mutations in their V_H genes and affinity maturation index with those induced with the help of WT T_{FH} cells (Fig. 4, D and E, and fig. S3A). Similar to what we observed in *Pdcd1*^{-/-} mice, the absolute number of PP T_{FH} cells was much higher in *Cd3e*^{-/-} mice that received *Pdcd1*^{-/-} T_{FH} cells than in mice injected with WT T_{FH} cells, and the *Pdcd1*^{-/-} T_{FH} cells maintained their skewed cytokine profile (Fig. 4F, and fig. S3, B and C). The results indicate that T_{FH} cells in *Pdcd1*^{-/-} mice preclude the generation of IgA-secreting cells in gut. On the other hand, both PD-1-deficient and sufficient Foxp3⁺ T cells, which, as previously reported (22), did not expand much but converted into IL-21-producing T_{FH} cells in PPs, supported the generation of LP IgAs after the transfer into *Cd3e*^{-/-} mice (Fig. 4, B, C, and F; and fig. S3, D and E). It was interesting that the IgAs induced by Foxp3⁺ T cells had significantly fewer mutations and low affinity maturation compared with those induced upon transfer of T_{FH} cells (Fig. 4, D and E). These observations suggest that a significant fraction of PCs in *Pdcd1*^{-/-} mice may be generated with the help provided by T_{FH} cells derived from Foxp3⁺ T cells.

Taken together, the results indicate that (i) the quality of gut IgAs critically depends on the number and the nature of T_{FH} cells in PPs; (ii) PD-1 deficiency interferes with the selection of B cells in GCs; and (iii) in addition to selection in PP GCs, IgAs appear to undergo a second, commensal-driven selection in the LP. Thus, the LP may serve not only as reservoir of PC but also as a site where proliferating PB are reselected to fit the geographical distribution of dynamic bacterial communities along the intestine.

In *Pdcd1*^{-/-} mice, as in SHM-defective AID^{G23S} mice (23), the gut microbiota induce hyperactivation of the systemic immune system (fig. S4, A and B). Indeed, along with T and B cell hyperplasia, we found that serum from *Pdcd1*^{-/-} mice contained antibodies specific for components of commensal bacteria, which indicated a breach of normal mucosal-systemic compartmentalization (fig. S4, A and C) (24). On the basis of the data presented here, we propose that the skewed gut microbial communities that result from the dysregulated selection of IgAs drive the expansion of self-reactive B and T cells. Our studies have implications for how modulation of PD-1 expression promotes tolerance or uncontrolled immune reactions leading to autoimmunity.

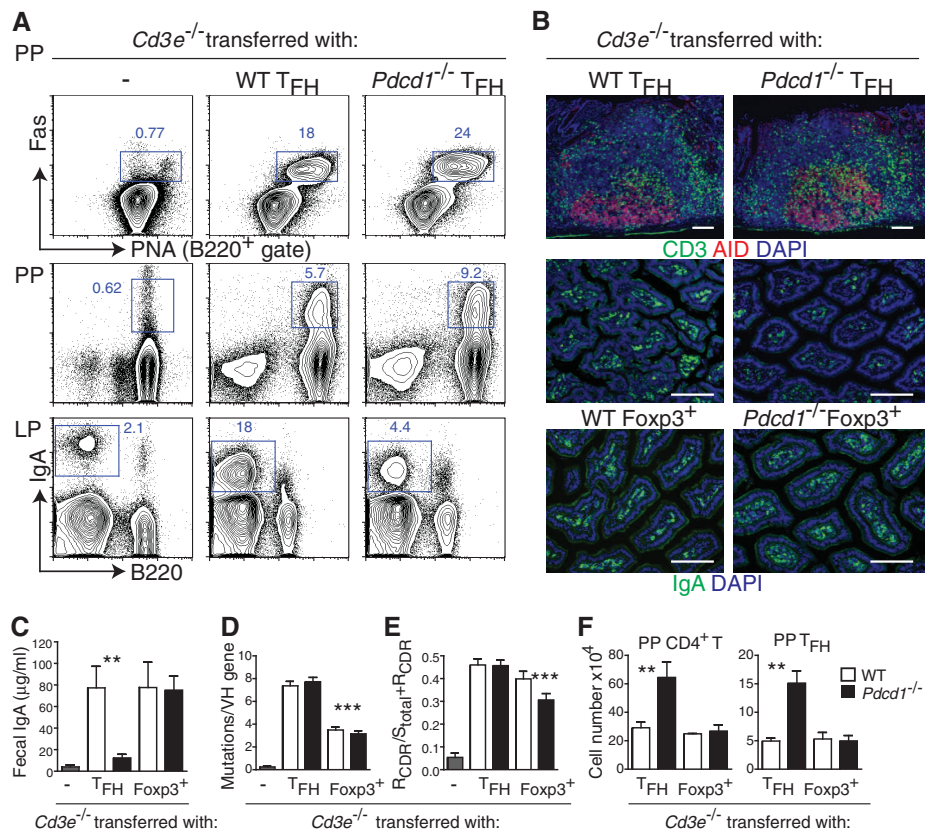


Fig. 4. T_{FH} cells from *Pdcd1*^{-/-} mice are impaired in their ability to support the generation of IgA plasma cells in gut. (A) Representative flow cytometric profiles of cells isolated from PPs and LP stained for the indicated markers. Numbers represent percentage of cells in the indicated gates. (B) Sections of the PPs and small intestine stained as indicated and (C) fecal IgA levels as determined by ELISA from the *Cd3e*^{-/-} mice 3 months after reconstitution with T_{FH} cells and Foxp3⁺ T cells from WT and *Pdcd1*^{-/-} mice. Scale bars, 100 μm. (D) Absolute number of somatic mutations in V_H genes and (E) the affinity maturation of the IgA-producing cells from the LP of *Cd3e*^{-/-} mice at 3 months after the reconstitution with the T cells indicated. The number of sequences analyzed: WT, T_{FH} = 171; *Pdcd1*^{-/-}, T_{FH} = 175; WT Foxp3⁺, T = 153; *Pdcd1*^{-/-} Foxp3⁺, T = 177. (F) Total numbers of indicated cells from the PPs of *Cd3e*^{-/-} mice at 3 months after the reconstitution with the T cells indicated. Means ± SEM (*n* = 2 to 3 mice per group). Two-tailed unpaired Student's *t* test was used for all statistical analyses; ****P* < 0.001; ***P* < 0.01.

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Supplementary Materials

www.sciencemag.org/cgi/content/full/336/6080/485/DC1
Materials and Methods
Figs. S1 to S4
References (25–34)

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An Essential Developmental Checkpoint for Production of the T Cell Lineage

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In early T cell development, progenitors retaining the potential to generate myeloid and natural killer lineages are eventually determined to a specific T cell lineage. The molecular mechanisms that drive this determination step remain unclarified. We show that, when murine hematopoietic progenitors were cultured on immobilized Notch ligand DLL4 protein in the presence of a cocktail of cytokines including interleukin-7, progenitors developing toward T cells were arrested and the arrested cells entered a self-renewal cycle, maintaining non-T lineage potentials. Reduced concentrations of interleukin-7 promoted T cell lineage determination. A similar arrest and self-renewal of progenitors were observed in thymocytes of mice deficient in the transcription factor Bcl11b. Our study thus identifies the earliest checkpoint during T cell development and shows that it is Bcl11b-dependent.

T cells are generated from multipotent hematopoietic stem cells through a series of differentiation steps. The first step in this pathway is the generation of progenitors that have lost erythroid/megakaryocyte potential but retain the capacity to generate other hemopoietic cells, including myeloid, T, and B cells (1–6). We and others have recently identified the next stage, in which the T cell progenitors have lost B cell potential but are still able to generate myeloid cells, dendritic cells (DCs), and natural killer (NK) cells (7, 8). Therefore, the most critical step for development of the T cell lineage is now thought to be at the point where myeloid potential is terminated.

We sought to identify the step at which progenitors become fully committed to the T cell lineage and what regulates this transition. A reliable way to substantiate that a given step is critical for the development of a lineage is to demonstrate developmental arrest at the stage before that step under particular conditions. In the case of B cell differentiation, deletion of *E2a*, *Ebf*, or *Pax5* genes leads to an early developmental arrest before formation of a functional *IgH* chain gene; these arrested B cell progenitors undergo self-renewal and remain B lineage uncommitted, with the potential to develop along other lineages, including myeloid and T cell (9–11). This case illustrates that such a critical developmental checkpoint exists at the step when uncommitted B cell progenitors become determined

to the B cell lineage. Unlike the B cell lineage, to date no such checkpoint has been identified for the T cell lineage before the initiation of *TCR* gene rearrangement.

As T cell progenitors develop, they proceed through developmental stages referred to as DN1 to DN4 (double-negative CD4[−]CD8[−]) that can be tracked by surface phenotype. The DN2 stage can be subdivided into two stages based on transgenic green fluorescent protein (GFP) expression controlled by the proximal *lck* (*plck*) promoter (*lck* is a *src* family kinase selectively expressed by T cells). GFP[−] cells retain non-T lineage potential, including that for myeloid cells, DCs, and NK cells, whereas the latter stage GFP⁺ cells are determined to the T cell lineage (7, 12). We designate these two stages DN2mt (myeloid-T) and DN2t (T-lineage determined) and term the step between these stages the DN2-determination step. This determination step is thought to be the first critical checkpoint in T cell development (13).

We cultured lineage-negative (Lin[−]) c-kit⁺ Sca-1⁺ (LKS) cells from 13 days post-coitum (dpc) murine fetal liver with immobilized Delta-like 4 (DLL4) protein in the presence of the cytokines SCF (stem cell factor), Flt3L (FMS-like tyrosine kinase ligand), and interleukin (IL)–7 (fig. S1). After 7 days of culture, cells remained at the DN stage (Fig. 1A, left panel), whereas in the control group, where cells were cultured with TSt-4 stromal cells expressing DLL4 (TSt-4/DLL4), generation of CD4⁺CD8⁺ double-positive (DP) cells was observed (fig. S2). Upon closer analysis on DN cells generated in the feeder-free condition, we observed that these cells resembled DN2mt cells (maintained c-kit^{high}CD25⁺) (Fig. 1A, right panel) and thus named them FFDN2 cells (feeder-free-cultured DN2-like cells). By several criteria, the FFDN2 cells appeared identical to DN2mt cells: (i) they gave rise to authentic αβ T cells when transferred to a TSt-4/DLL4 stromal cocul-

ture system (fig. S3, A and B); (ii) they retained the potential to produce macrophages (Fig. 1B), NK cells, and DCs (fig. S3, C and D); (iii) intracellular T cell receptor (TCR) β chain protein was not expressed (Fig. 1C); and (iv) their gene expression profiles were similar to those of DN2mt cells (Fig. 1D and fig. S4). Furthermore, GFP expression was not observed in FFDN2 cells generated from progenitors isolated from *plck*-GFP mice (Fig. 1E). It is unlikely that this arrest is due to the failure of TCR gene rearrangement because enforced expression of a functional TCRβ chain gene did not prevent the developmental arrest (fig. S5). FFDN2 cells could not generate B cells (fig. S3E), indicating that dedifferentiation to more primitive progenitors did not occur in this culture system. Of note, FFDN2 cells showed an almost unlimited in vitro expansion (Fig. 1F), while essentially maintaining c-kit and CD25 expression (Fig. 1G) and a developmental potential comparable to that of freshly isolated DN2mt cells (fig. S6). Cells in the c-kit⁺CD25⁺ fraction possessed the potential to maintain long-term culture, because long-term culture could be maintained by using c-kit⁺CD25⁺ cells at the time of passage (fig. S7). Such self-renewal capacity, together with our other results, indicated that the DN2-determination step may be a critical checkpoint for T cell development.

To investigate the molecular mechanisms of T cell lineage determination, we searched for an environmental cue that could drive the arrested cells through the DN2-determination step. After testing various cytokines and Notch ligand conditions in the feeder-free culture system, we found that FFDN2 cells initiate differentiation when the concentration of IL-7 is reduced on day 7 of culture (10 ng/ml to 1 ng/ml). In this induction system, GFP⁺ DN3 cells appear on day 3 after IL-7 reduction (Fig. 2A). These cells did not express myeloid-lineage transcription factors *PU.1* (*Spi1*) and *C/EBPα*, whereas T cell lineage-associated genes such as *lck*, *Tcf1*, *pTa*, and *Bcl11b* were markedly up-regulated (Fig. 2B). Notably, cells in these cultures developed up to the αβTCR-expressing CD4⁺CD8⁺ DP stage (Fig. 2C and figs. S8 and S9). Although the kinetics of DP cell growth was delayed compared with that in the TSt-4/DLL4 feeder cell culture system, the final yield of DP cells was nearly identical (fig. S10). The DP cells generated by reducing the concentration of IL-7 appeared to be authentic DP cells, because they give rise to CD4 and CD8 single-positive (SP) cells when transferred to a fetal thymus organ culture system (fig. S11). These results demonstrated that αβTCR⁺ cells can be generated from prethymic progenitors in a “feeder-free” culture system and that the TCRβ-selection, which is thought to serve as the critical checkpoint for preTCR formation in progenitors, does not require additional environmental factors in this feeder-free culture system.

Often transcription factors regulate cell lineage determination steps. Among genes up-regulated by our induction system, we focused on *Bcl11b*, a T cell lineage-specific transcription factor origi-

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Fig. 1. Immobilized DLL4 with a combination of cytokines induces self-renewing expansion of immature thymocytes. **(A)** LKS progenitor cells (200 cells) from 13 dpc murine fetal liver were cultured with immobilized Fc-DLL4 supplemented with 10 ng/ml of SCF, IL-7, and Flt3L for 7 days. Generated cells were harvested and stained with the indicated antibodies and analyzed by a flow cytometry. Data are representative of four independent experiments. **(B)** A photomicrograph of macrophages generated from FFDN2 cells. FFDN2 cells induced from green mouse progenitors in a similar manner as (A) were sorted and cultured with Tst-4 stromal cells for 14 days in the presence of 10 ng/ml M-CSF (macrophage colony-stimulating factor). Macrophages are seen as large GFP⁺ cells. Scale bar, 100 μ m. **(C)** Expression of intracellular (ic) TCR β in FFDN2 cells, in negative control cells generated in feeder-free culture using only the Fc portion (Control), and in positive control CD4⁺CD8⁺ DP cells from an adult thymus. **(D)** mRNA expression of lineage-specific genes in cells derived from DN1, DN2mt, DN3, DP, and FFDN2 cells determined by quantitative reverse transcription polymerase chain reaction (RT-PCR). Expression was normalized to acidic ribosomal protein (ARP) mRNA expression, and the mean $\pm$ SD of triplicate samples is shown. Data are representative of three independent experiments. **(E)** Flow cytometric analysis of GFP expression in FFDN2 cells generated from progenitors of plck-GFP mice in comparison with cells generated under Tst-4/DLL4 conditions (Control). Data are representative of three independent experiments. **(F)** A growth curve of FFDN2 cells. Viable cells were enumerated at the indicated time points. **(G)** c-kit versus CD25 expression by FFDN2 cells after long-term culture. Fetal liver LKS cells were cultured under feeder (–) conditions for 30 days and then analyzed by flow cytometry. Data are representative of four independent experiments.

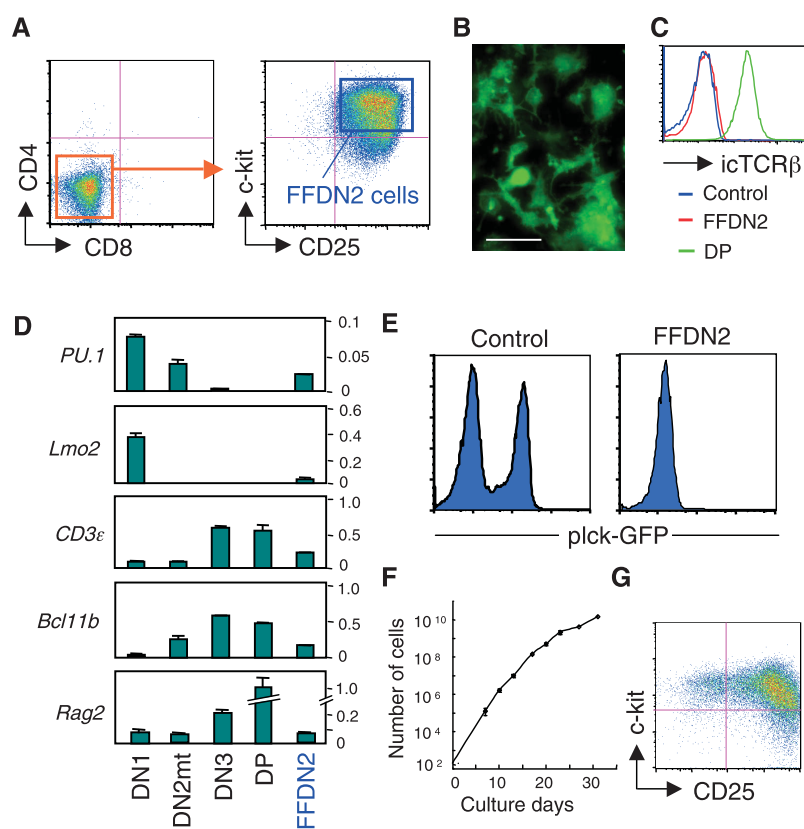
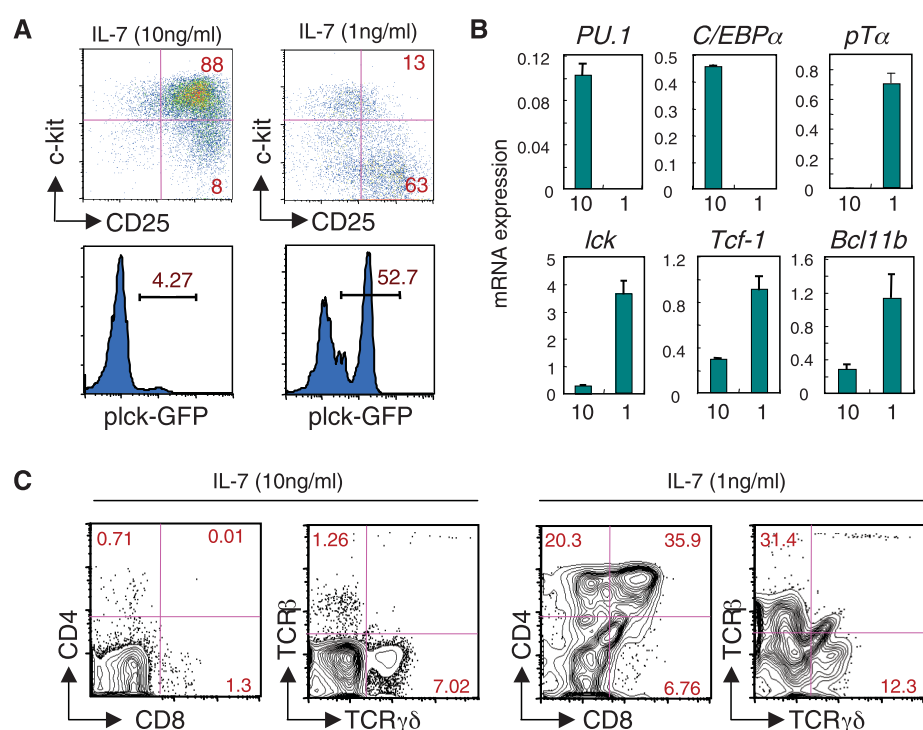


Fig. 2. Reduction of IL-7 concentration induces the generation of DP cells in feeder-free culture. **(A)** LKS cells (200 cells) from 13 dpc fetal liver were cultured with immobilized Fc-DLL4 in the presence of SCF, IL-7, and Flt3L (10 ng/ml). After 7 days, the concentration of IL-7 was maintained or reduced to 1 ng/ml, and the cells were cultured for an additional 3 days. Cells were analyzed by flow cytometry. Data are representative of three independent experiments. **(B)** mRNA expression of lineage-associated genes in cells cultured in the presence of 10 ng/ml or 1 ng/ml of IL-7 in the same manner as (A). Expression was normalized to ARP mRNA expression, and the mean $\pm$ SD of triplicate samples is shown. Data are representative of three independent experiments. **(C)** Flow cytometric analysis of cells generated 7 days after switching to cultures with either high or low IL-7 concentration. Cells were analyzed for the expression of CD4 versus CD8 and TCR β versus TCR $\gamma\delta$. Data are representative of five independent experiments.



nally identified as a tumor suppressor in murine T cell lymphoma (14). *Bcl11b*-deficient mice exhibit impaired thymocyte development around the DN3 to immature SP stage because of an inability to rearrange the V_{β} to D_{β} gene segments (15). We carefully reexamined the phenotype of fetal thymus cells from *Bcl11b*-deficient mice and found that, at 18 dpc, there was a developmental

arrest at the DN2 stage (Fig. 3A). Despite this, the absolute number of DN2 cells was not increased (Fig. 3B), indicating that self-renewing expansion is not so prominent in vivo, a difference that could be due to the limited niche space in the thymus for early progenitors. We cultured these DN2 cells under Tst-4/DLL4 conditions, which can support T cell differentiation up to the DP stage. In such

cultures, *Bcl11b*^{-/-} cells continued to proliferate even after 4 weeks, maintaining their DN2 surface phenotype (Fig. 3C). Similar to FFDN2 cells, *Bcl11b*^{-/-} DN2 cells exhibited features of DN2mt cells, including the potential to develop into macrophages and NK cells (Fig. 3D), and loss of B cell potential (fig. S12).

Bcl11b deficiency is lethal around the neonatal period (15). To investigate whether the developmental arrest of *Bcl11b*^{-/-} progenitors is seen in the adult thymus, where T cells are continuously generated, we produced chimeric mice by transferring *Bcl11b*^{-/-} fetal liver cells into irradiated B6Ly5.1 congenic mice. At 8 weeks after transfer, we observed nearly complete developmental arrest at the DN2 stage, with only a few DP cells (Fig. 3E). Similar to ex vivo fetal thymocytes of *Bcl11b*^{-/-} mice and cultured *Bcl11b*^{-/-} DN2 cells, the arrested DN2 cells were equivalent to DN2mt cells. There was no increase in thymic B cells in the recipients of the *Bcl11b*^{-/-} fetal liver cells (fig. S13), indicating that the *Bcl11b*^{-/-} DN2 cells that developed in the thymus did not dedifferentiate into more primitive progenitors in vivo.

The similar stage of arrest in the DLL4/IL-7 cultures and in the *Bcl11b*^{-/-} mice suggested that the arrest in the cultures may be due to a failure to up-regulate *Bcl11b*. To examine this possibility, we retrovirally transduced *Bcl11b* cDNA into fetal liver LKS cells and cultured these transduced cells under DLL4/IL-7 conditions. The *Bcl11b*-transduced cells could give rise to DN3 cells even in the presence of a high concentration of IL-7 (Fig. 4A), and TCR β gene rearrangement was enhanced (Fig. 4B), whereas myeloid-lineage-associated genes were suppressed (Fig. 4C), demonstrating that *Bcl11b* expression eliminated the DN2 arrest that occurred in the DLL4/IL-7 cultures.

As has been reported (16), the absence of *Bcl11b* had a severe impact on the generation of thymic $\alpha\beta$ T cells, whereas there was little effect on the generation of $\gamma\delta$ T cells (fig. S14A). The same is true for cells generated in the DLL4/IL-7 cultures (fig. S14B). These results suggested that the segregation to the $\gamma\delta$ T cell lineage occurs before the DN2mt stage, although the possibility still remains that the $\gamma\delta$ T cells that had been generated from "leaky" DN3 cells underwent compensatory proliferation.

The developmental steps just after the formation of preTCR (DN3 stage) and $\alpha\beta$ TCR (DP stage) serve as critical checkpoints (16, 17), and cells that fail to pass these points succumb to apoptotic cell death. In contrast, the arrested progenitors at the DN2-determination step enter a self-renewal cycle. The appearance of self-renewing progenitors among *Bcl11b*^{-/-} thymocytes may explain the previous findings that loss-of-function mutations in the *Bcl11b* gene are frequently observed in murine T cell lymphomas induced by γ irradiation (14) and that chromosomal aberration disrupting *Bcl11b* gene was identified in human T cell acute lymphoblastic

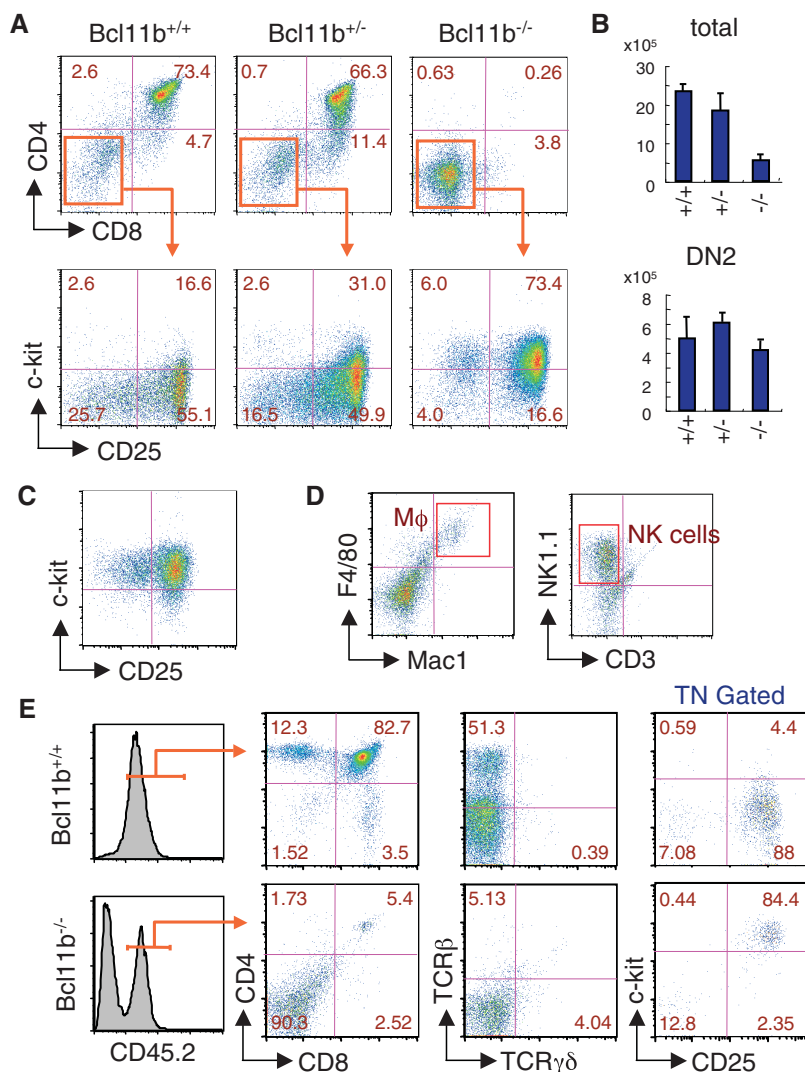
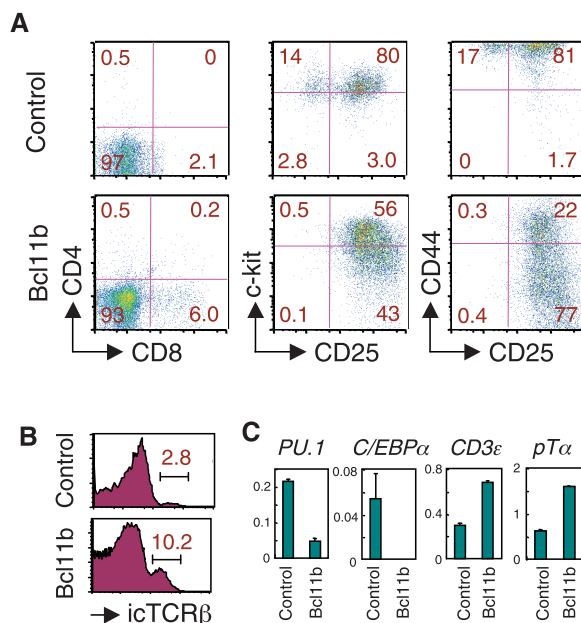


Fig. 3. *Bcl11b* is essential for T cell lineage determination. (A) Flow cytometric analysis of fetal thymocytes from *Bcl11b*^{-/-} mice. Profiles are shown for CD4 versus CD8 of 18 dpc fetal thymocytes, and c-kit versus CD25 of cells gated in upper panels, from the indicated mice. For each group, more than five mice were individually analyzed, and representative profiles are shown. (B) Absolute numbers of total thymocytes and DN2 cells in 18 dpc fetuses of the indicated *Bcl11b* genotypes. More than five mice were individually analyzed for each group, and the mean + SD is shown. (C) Flow cytometric analysis of c-kit versus CD25 of fetal liver LKS cells from *Bcl11b*^{-/-} mice cultured on Tst-4/DLL4 stromal cells for 30 days. Data are representative of three independent experiments. (D) Generation of macrophages and NK cells from cultured *Bcl11b*^{-/-} fetal liver cells. The c-kit⁺CD25⁺ cells shown in (C) were cultured (200 cells per well) for 7 days with Tst-4 cells in the presence of M-CSF (left panel) or IL-15 (right panel) and analyzed for macrophage and NK cell markers by flow cytometry. Data are representative of three independent experiments. (E) Fetal liver cells from *Bcl11b*^{+/+} or *Bcl11b*^{-/-} mice (Ly5.2) were transferred into lethally irradiated mice (Ly5.1). Flow cytometric profiles of reconstituted thymocytes of recipient mice 8 weeks after transfer are shown. In right panels, profiles of cells gated on CD3⁻CD4⁻CD8⁻ [triple negative (TN)] fraction are shown. For each group, more than five mice were individually analyzed, and representative data are shown.

Fig. 4. Enforced expression of Bcl11b abrogated the DN2 arrest in the DLL4/IL-7 cultures. **(A to C)** LKS cells from 13 dpc B6 fetal liver were transduced with murine stem cell virus (MSCV)-Bcl11b or MSCV-control vector. Two days later, GFP⁺ cells were sorted and cultured with immobilized Fc-DLL4 in the presence of 10 ng/ml of SCF, IL-7, and Flt3L for 7 days. Flow cytometric profiles of CD4 versus CD8, c-kit versus CD25, and CD44 versus CD25 expression **(A)**, iCTCR β expression **(B)**, and mRNA expression **(C)** in generated cells are shown. Expression of mRNA was normalized to ARP mRNA expression, and the mean + SD of triplicate samples is shown. Data are representative of three independent experiments.



leukemia cases (18), because the acquisition of self-renewal capacity is regarded as the first step in leukemia development. In this context, a similar outcome was recently observed when *Lmo2*, a known oncogene, was overexpressed in thymocytes and caused the cells to enter a self-renewal cycle in vivo (19).

The present study thus defines a Bcl11b-driven checkpoint at which T cell progenitors terminate non-T-lineage potential in order to become determined to the T cell lineage (fig. S15). Our finding that Bcl11b up-regulation can be triggered by an extrinsic cue, diminished IL-7, suggests that progression through the DN2-determination step is instructed by environmental

signals in the thymus. It is quite likely that the reduction in IL-7 signaling is a physiological mediator of this step, because the IL-7R is dramatically down-regulated at the transition from the DN2 to the DN3 stage (20). Considering that Bcl11b is thought to be a transcriptional repressor, we speculate that Bcl11b directly suppresses myeloid-lineage-associated genes, such as *PU.1* or *C/EBP α* , and that such suppression is critical for differentiation toward the T cell fate.

A recent study demonstrated that Bcl11b is expressed in the T cell-like lymphoid cells of lamprey (21). Because a Bcl11b homolog has not been found in animals other than vertebrates (fig. S16), we propose that Bcl11b arose in phy-

logeny to construct a new lineage distinct from the preexisting innate type killer cells.

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Supporting Online Material

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Materials and Methods

Figs. S1 to S16

References

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Repression of the Transcription Factor Th-POK by Runx Complexes in Cytotoxic T Cell Development

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Mouse CD4⁺CD8⁺ double-positive (DP) thymocytes differentiate into CD4⁺ helper-lineage cells upon expression of the transcription factor Th-POK but commit to the CD8⁺ cytotoxic lineage in its absence. We report the redirected differentiation of class I-restricted thymocytes into CD4⁺CD8[−] helper-like T cells upon loss of Runx transcription factor complexes. A Runx-binding sequence within the *Th-POK* locus acts as a transcriptional silencer that is essential for *Th-POK* repression and for development of CD8⁺ T cells. Thus, Th-POK expression and genetic programming for T helper cell development are actively inhibited by Runx-dependent silencer activity, allowing for cytotoxic T cell differentiation. Identification of the transcription factors network in CD4 and CD8 lineage choice provides insight into how distinct T cell subsets are developed for regulating the adaptive immune system.

The peripheral T cell repertoire is formed after developing thymocytes have undergone a series of developmental selection processes. CD4⁺CD8⁺ double-positive (DP) thymocytes

undergo positive selection through T cell receptor (TCR) interaction with major histocompatibility complex (MHC) proteins. This gives rise to two functionally distinct subsets:

CD4⁺CD8[−] helper and CD4[−]CD8⁺ cytotoxic T cells. Cells expressing MHC class II-restricted TCRs differentiate into the helper lineage and cease CD8 expression, whereas cells expressing class I-restricted TCRs differentiate into the cytotoxic lineage and silence CD4 expression (1–3). Recently, gain or loss of function of the BTB/POZ domain-containing zinc finger transcription factor, Th-POK, revealed that its expression is essential and sufficient for development of helper-lineage cells (4, 5).

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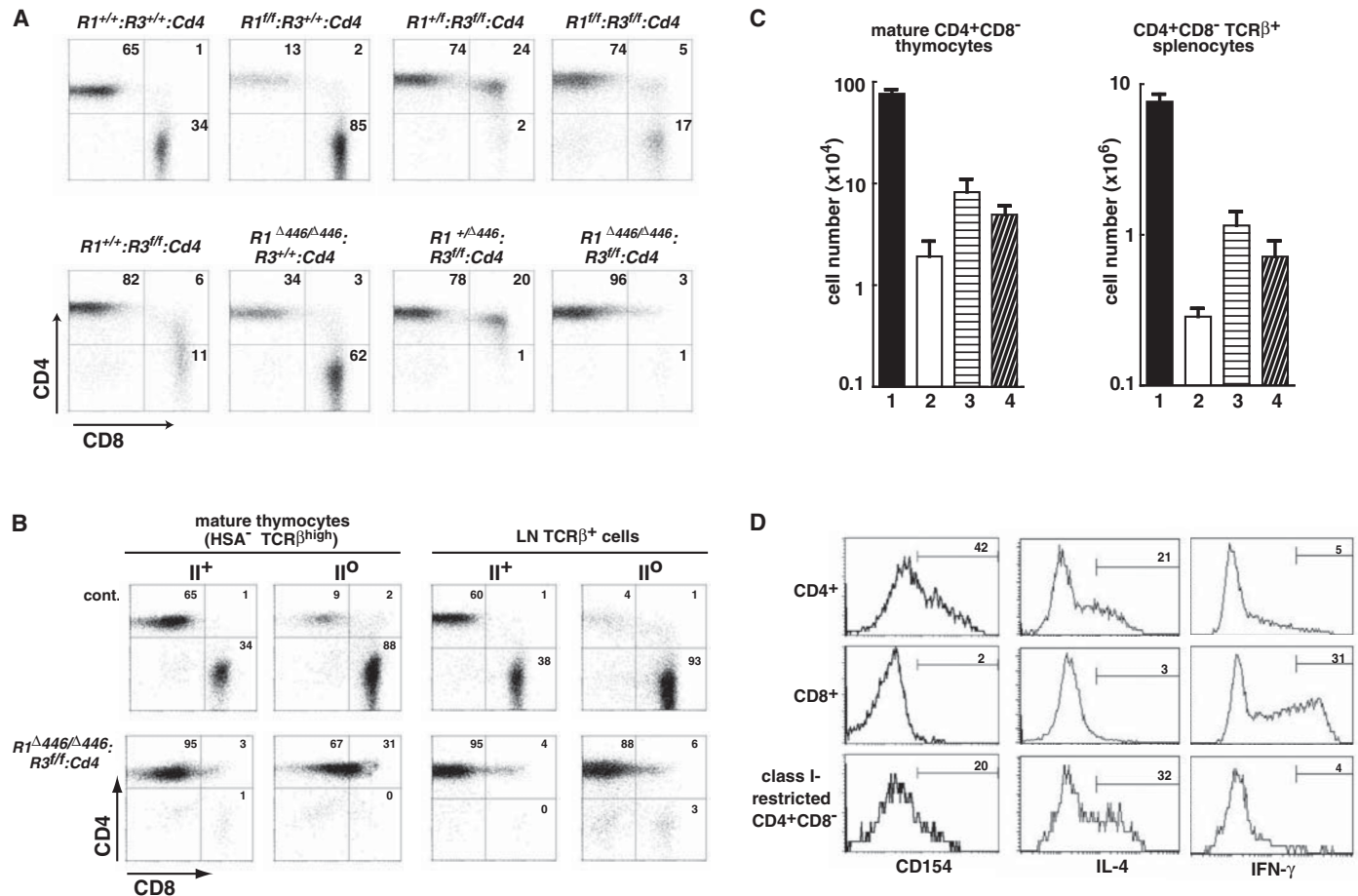


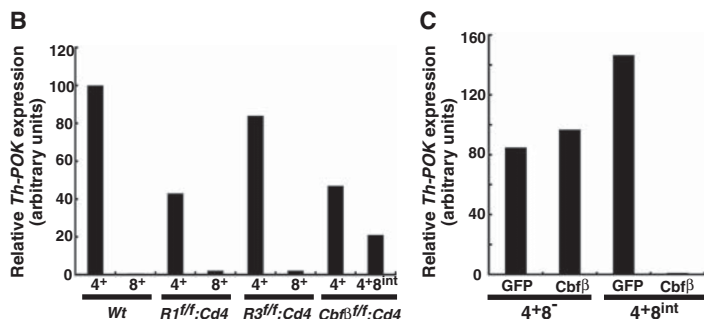
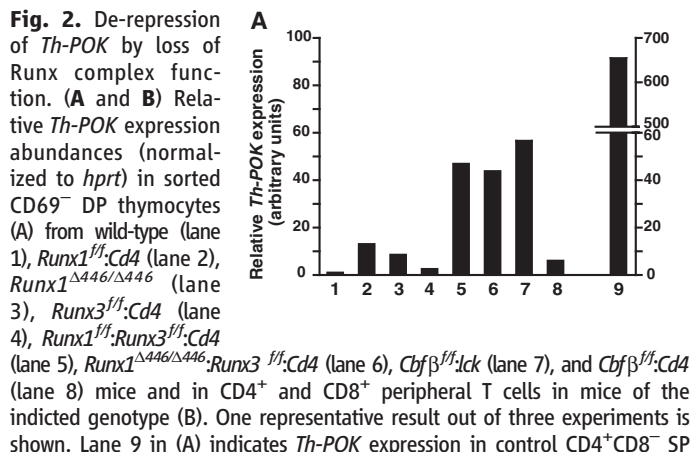
Fig. 1. Differentiation of class I-restricted cells into CD4⁺CD8[−] helper-like cells by loss of Runx complex function. **(A)** CD4 and CD8 expression in lymph node $\alpha\beta$ T cells from mice with indicated genotypes. **(B)** CD4 and CD8 expression in mature thymocytes and LN TCR β^{+} T cells either in the presence (II⁺) or absence (II^o) of I-A MHC class II molecules. **(C)** Cell numbers of mature thymocytes and splenocytes showing CD4⁺CD8[−] $\alpha\beta$ T cells in class II⁺ control mice (lane 1), class II^o control mice (lane 2), class II⁺ $Runx1^{\Delta446/\Delta446};Runx3^{fl/fl};Cd4$

mice (lane 3), and class II^o $Runx1^{\Delta446/\Delta446};Runx3^{fl/fl};Cd4$ mice (lane 4). Error bars indicate standard deviation. **(D)** Expression of CD154 at 42 hours after in vitro TCR stimulation of control CD4⁺, CD8⁺, and class I-restricted CD4⁺CD8[−] cells. Intracellular staining of IL-4 and IFN- γ analyzed at 6 hours after re-stimulation of cells that were cultured for 5 days after initial TCR stimulation. Numbers in the plots in (A), (B), and (D) indicate the percentage of cells in each quadrant or region.

Runx transcription factor complexes are composed of heterodimers for one of three Runx proteins and their obligatory non-DNA-binding partner, Cbfb protein (6). Because of the embryonic or neonatal lethality of mice deficient for any of Runx family genes, we used the Cre/loxP-mediated conditional gene inactivation (7) to clarify Runx complex function in silencing of

the *Cd4* gene (8) and recently reported that the combined inactivation of *Runx1* and *Runx3* in DP thymocytes resulted in a dramatic loss of CD8⁺ T cells (9). Runx proteins possess a conserved Val-Trp-Arg-Pro-Tyr (VWRPY) motif at the C-terminal end, allowing the recruitment of the Groucho/TLE co-repressor proteins to their target genes (10, 11). To test whether VWRPY-

dependent repression might be involved in the loss of CD8⁺ T cells, we introduced the *Runx1*^{Δ446} allele (12) that generates a mutant Runx1 protein lacking the VWRPY motif on a Runx3-deficient background (*Runx3*^{fl/fl}:*Cd4* mice) (13). A marked reduction of splenic CD8⁺ T cells in *Runx1*^{Δ446/Δ446}:*Runx3*^{fl/fl}:*Cd4* mice (Fig. 1A and fig. S1) indicated that VWRPY-dependent repres-



thymocytes. (C) Relative *Th-POK* expression abundances after reconstitution of Runx complex function. Purified CD4⁺CD8⁻ and CD4⁺CD8^{int} cells from *Cbfb*^{fl/fl}:*Cd4* mice were transduced with control retroviral vector (GFP) or vector encoding Cbfb (Cbfb).

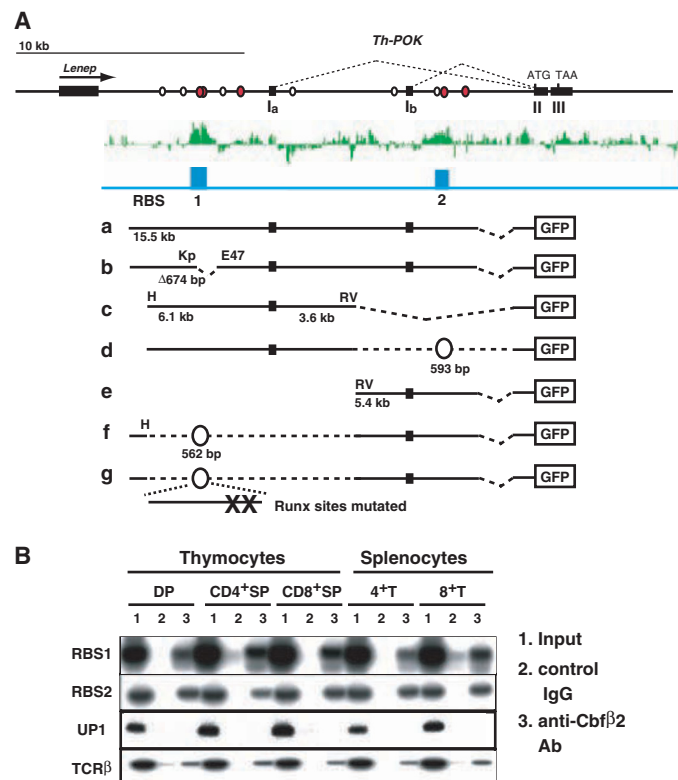
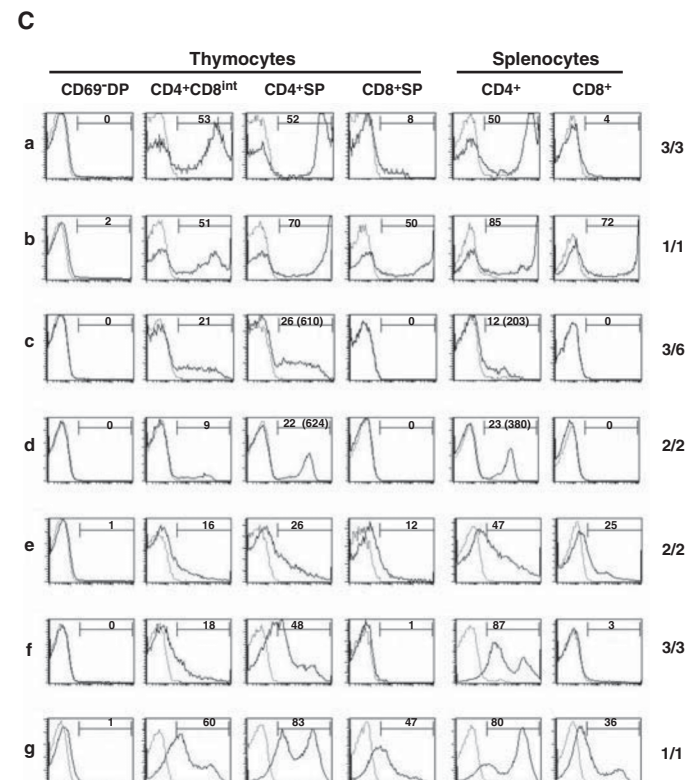


Fig. 3. Identification and characterization of RBSs at the *Th-POK* locus. (A) The structure of the murine *Th-POK* locus is shown at the top. Circles represent putative Runx motifs, with those in red indicating evolutionarily conserved Runx motifs. Black boxes represent exons, and each green bar represents the signal intensity of an individual oligonucleotide probe in a ChIP-on-chip experiment. Blue boxes represent RBSs. The maps for each reporter transgene construct (Tg-a to Tg-g) are indicated. The restriction sites shown are Eco47III (E47), EcoRV (RV), HindIII (H), KpnI (Kp), and XhoI (X). (B) ChIP experiment showing binding of Runx complexes to RBS-1 and RBS-2 in



the indicated cell subsets. The regions at 1 kb upstream of exon Ia (UP1) and the TCRβ enhancer (TCRβ) were used as negative and positive controls, respectively. (C) Histograms showing the GFP expression in the indicated T cell subsets from representative transgenic founder for each construct. The dashed line indicates nontransgenic littermate control. Numbers in the histogram indicate the percentage of GFP⁺ cells, and numbers in parenthesis indicate mean fluorescent intensity of GFP in GFP⁺ cells. The numbers of transgenic founders expressing GFP among the total transgenic founders are indicated at right.

sion by Runx1 was involved in the generation of CD8⁺ T cells. Because the leaky CD4⁺CD8⁺ subset that escaped Cre-mediated recombination (9) was less apparent in *Runx1*^{Δ446/Δ446};*Runx3*^{fl/fl};*Cd4* mice (Fig. 1A), we used these mice for further analyses.

Potentially, the loss of CD8⁺ T cells could occur either by a developmental block of class I-restricted cells or by a redirection of class I-restricted cells toward the CD4⁺CD8⁺ lineage. To determine whether CD4⁺CD8⁺ cells that emerge in Runx mutant mice are class II-restricted or redirected class I-restricted cells, we crossed *Runx1*^{Δ446/Δ446};*Runx3*^{fl/fl};*Cd4* mice onto a MHC class II-deficient background (14). Although there was a marked decrease in CD4⁺CD8⁺ T cell numbers in control class II-deficient mice, the predominance of CD4⁺CD8⁺ T cells persisted in class II-deficient *Runx1*^{Δ446/Δ446};*Runx3*^{fl/fl};*Cd4* mice in both the thymus and the periphery (Fig. 1, B and C). These results indicated that the absence of Runx complexes forced the majority of class I-restricted cells to differentiate into CD4⁺CD8⁺ T cells.

We next examined the functional properties of these CD4⁺CD8⁺ cells. One of the characteristic features of CD4⁺ helper-lineage T cells is the early induction of CD154, the ligand for CD40, after TCR stimulation (15) and the production of interleukin-4 (IL-4). These were observed in control CD4⁺ T cells as well as in class I-restricted CD4⁺CD8⁺ cells, but not in control CD8⁺ T cells (Fig. 1D). In contrast, although high interferon-γ (IFN-γ) production was detected in control CD8⁺ T cells, it was absent in both wild-type CD4⁺ T cells and in class I-restricted CD4⁺CD8⁺ cells

(Fig. 1D). We conclude from these results that class I-restricted CD4⁺CD8⁺ cells that develop in Runx mutant animals are functionally helper-like T cells.

Because ectopic expression of Th-POK has been shown to redirect class I-restricted cells to become CD4⁺CD8⁺ cells (4, 5), we measured the expression of *Th-POK* in several Runx mutant mice, including a strain in which the *Cbfb* gene is conditionally inactivated by either a *Lck-Cre* or a *Cd4-Cre* transgene (13). Consistent with a previous report (4), *Th-POK* expression was not detected in control CD69⁺ DP thymocytes. In contrast, a 40-fold increase in *Th-POK* transcript abundances was detected in CD69⁺ DP thymocytes in which Runx complexes were disrupted either by combined Runx1 mutations with a Runx3 deficiency or by loss of Cbfb protein (*Cbfb*^{fl/fl};*Lck* mice) (Fig. 2A). A modest *Th-POK* de-repression by inactivation of *Runx1* alone indicated a redundant function of Runx3 in the repression of *Th-POK*.

Although *Th-POK* mRNA was undetectable in control CD8⁺ T cells and in CD8⁺ T cells deficient for Runx1 or Runx3, it was present in Cbfb-deficient CD4⁺CD8⁺ T cells (Fig. 2B) that still developed in *Cbfb*^{fl/fl};*Cd4* mice because of the slow turnover of Cbfb protein after inactivation of the *Cbfb* gene (13). We therefore next examined whether *Th-POK* repression could be restored in these CD4⁺CD8⁺ cells upon re-expression of Cbfb protein. Purified CD4⁺CD8⁺ and CD4⁺CD8⁺ cells were transduced with a retroviral vector encoding Cbfb or with an empty vector control. In these experiments, expression of *Th-POK* was markedly reduced upon re-

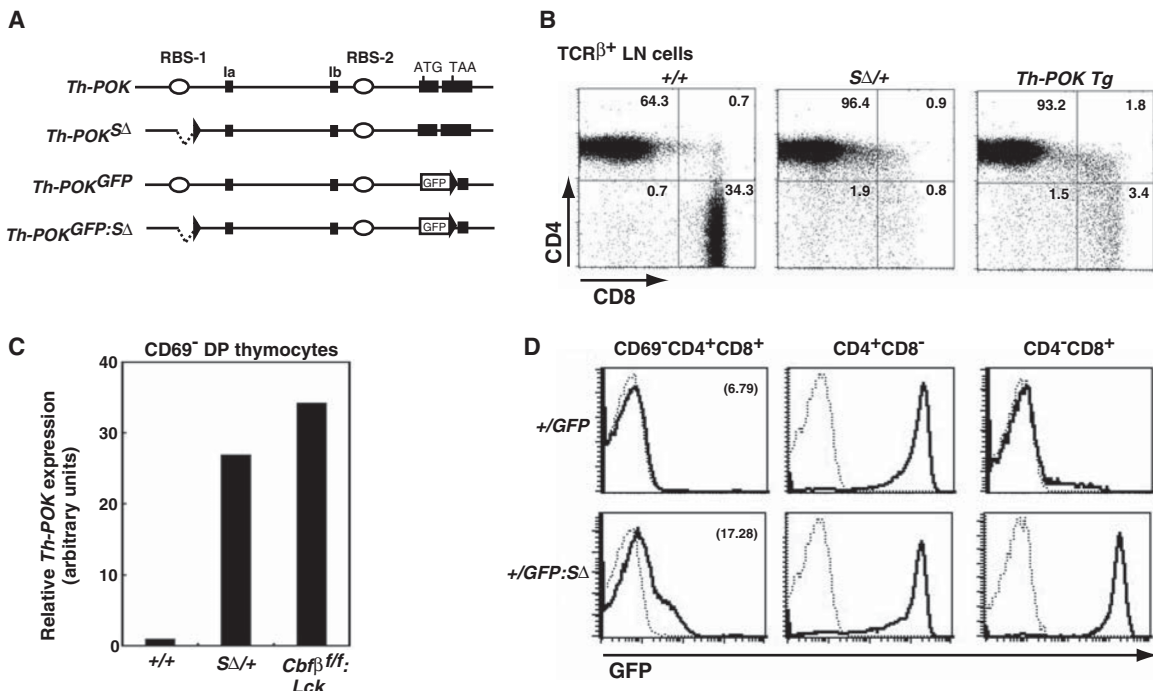
expression of Cbfb in CD4⁺CD8⁺ cells, with no detectable effect in CD4⁺CD8⁺ cells (Fig. 2C). These results suggest that Runx-mediated *Th-POK* repression operates in peripheral CD8⁺ T cells.

To understand mechanisms underlying Runx-mediated repression of *Th-POK*, we examined whether Runx complexes directly associate with the *Th-POK* locus. Using a ChIP-on-chip (ChIP indicates chromatin immunoprecipitation) approach with an antibody against Cbfb2, we detected two regions occupied by Runx complexes within the *Th-POK* locus. Distal and proximal Runx-binding sequences (RBS-1 and RBS-2, respectively) are located ~3.1 kb upstream and ~7.4 kb downstream of exon 1a (Fig. 3A) and contain two or one conserved Runx motifs, respectively (Fig. 3A and figs. S2 and S3). By using ChIP analysis in T cell subsets, we confirmed an association between Runx complexes and these two regions (Fig. 3B). However, binding of Runx complexes to RBS-1 and RBS-2 was detected in both *Th-POK*-expressing and nonexpressing cells, revealing that the binding of Runx complexes to these regions did not correspond with *Th-POK* repression.

To better understand the functional activities of RBS-1 and RBS-2 in light of these results, we performed transgenic reporter assays. A 15.5-kb genomic fragment encompassing the RBSs and exons 1a and 1b was linked to a green fluorescent protein (GFP) reporter transgene cassette (Tg-a in Fig. 3A). In all transgenic mouse founders obtained with Tg-a, GFP expression was first detected in post-selection CD4⁺CD8⁺ thymocytes and was up-regulated in CD4⁺ SP thymocytes, remaining

Fig. 4. Essential require-

ment of the *Th-POK* silencer for development of CD8⁺ T cells. (A) Schematic structure of the *Th-POK* locus and targeted alleles *Th-POK*^{ΔΔ}, *Th-POK*^{GFP}, and *Th-POK*^{GFP:ΔΔ}. Exons and loxP sequences are indicated as black boxes and black triangles, respectively. (B) CD4 and CD8 expression in lymph node αβT cells from wild-type (+/+), *Th-POK*^{ΔΔ} heterozygous (*SΔ*/+), and *Th-POK* hemizygous transgenic (*Th-POK* Tg) mice. (C) Relative *Th-POK* expression abundances in sorted CD69⁺ DP thymocytes showing de-repression of *Th-POK* upon deletion of the *Th-POK* silencer. (D) GFP expression from the *Th-POK*^{GFP} and *Th-POK*^{GFP:ΔΔ} alleles in indicated thymocyte subsets. Dashed and bold lines indicate GFP expression in control mice and *Th-POK*^{+/GFP} (+/GFP) or *Th-POK*^{+/GFP:ΔΔ} (+/GFP:ΔΔ) mice, respectively. The numbers in parenthesis indicate mean fluorescent intensity of GFP in total CD69⁺ DP thymocytes.



high in splenic CD4⁺ T cells, whereas it was almost undetectable in splenic CD8⁺ T cells (Fig. 3C). The 15.5-kb fragment thus contains the major *cis*-regulatory regions that direct expression of *Th-POK* in the helper lineage.

To further narrow down the critical *Th-POK* regulatory regions, we deleted either 5' or 3' sequences as well as RBS-1 from the 15.5-kb fragment. Whereas RBS-2 (fig. S3) was found to be required for positive transcriptional regulatory activity (as in the Tg-c and Tg-d constructs), deletion of a 674-bp fragment of RBS-1 (Tg-b) resulted in GFP expression both in CD4⁺ helper-lineage and in CD8⁺ cytotoxic-lineage cells, indicating that RBS-1 is a transcriptional silencer required to repress the reporter gene in CD8 lineage cells. Efficient repression of GFP in CD8⁺ T cells by a 562-bp fragment of RBS-1 (fig. S2) in the context of Tg-e construct required Runx motifs (Tg-f and Tg-g) (Fig. 3C), consistent with Runx-dependent activity of RBS-1 silencer.

To examine the physiological function of the RBS-1 silencer, we deleted the 674-bp KpnI-Eco47III sequences from the *Th-POK* locus by homologous recombination in embryonic stem (ES) cells (Fig. 4A and fig. S4). Deletion of RBS-1 from one *Th-POK* allele led to the loss of peripheral CD8⁺ T cells (Fig. 4B) and to the *Th-POK* de-repression in CD69⁺ DP thymocytes (Fig. 4C). We further investigated *Th-POK* de-repression by using mice in which the coding sequence for Th-POK was replaced with the *gfp* gene (*Th-POK*^{GFP} locus). GFP expression in mice heterozygous for *Th-POK*^{GFP} allows us to examine expression of *Th-POK* at the single-cell level. Although GFP expression from the *Th-POK*^{GFP} locus was not detected in CD69⁺ DP thymocytes, deletion of RBS-1 (*Th-POK*^{GFP:Δ} locus in Fig. 4A) resulted in uniform de-repression of GFP in CD69⁺ DP thymocytes, followed by

high GFP expression in both helper- and cytotoxic-lineage mature thymocytes (Fig. 4D).

Our results reveal that helper lineage-specific expression of *Th-POK* is regulated by the RBS-1 silencer, whose activity depends on binding of Runx complexes. We therefore refer to RBS-1 as the *Th-POK* silencer (fig. S5). The association of Runx complexes with the *Th-POK* silencer in cells expressing *Th-POK* indicates that specificity of silencer activity is not regulated at the level of Runx complex binding. Additional molecules that interact with Runx factors bound to the *Th-POK* silencer may therefore have a central role in regulating *Th-POK* silencer activity.

The antagonistic interplay between primary lineage-determining factors is often observed when two opposing fates are induced in progenitor cells (16, 17). Th-POK was recently described as an inhibitor of Runx-dependent *Cd4* silencer activity (18), consistent with an antagonistic interplay between these two factors. Identification of Th-POK and Runx complex target genes will help to further unravel the transcription factors network regulating lineage specification of DP thymocytes.

Uniform de-repression of Th-POK in CD69⁺ DP thymocytes upon deletion of the *Th-POK* silencer indicates that silencer-mediated *Th-POK* repression operates in all pre-selection DP thymocytes. It is therefore possible that TCR signals after engagement of MHC class II result in antagonism of *Th-POK* silencer activity and thus induce *Th-POK* expression. Given that sustained class II-specific TCR signals are thought to be necessary for specification of the helper lineage (19–21), reversal of silencer-mediated *Th-POK* repression may require class II-specific TCR signals during a specified time window. Our results suggest that a mechanism regulating *Th-POK* silencer activity acts as a sensor to dis-

tinguish qualitative differences in TCR signaling. Further studies on the regulatory pathways of *Th-POK* repression will shed light on how signals initiated by external stimuli are converted into genetic programs in the cell nucleus.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/319/5864/822/DC1

Material and Methods

Figs. S1 to S5

References

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PDLIM2 Inhibits T Helper 17 Cell Development and Granulomatous Inflammation Through Degradation of STAT3

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Granuloma formation is an important host defense mechanism against intracellular bacteria; however, uncontrolled granulomatous inflammation is pathologic. T helper 17 (T_H17) cells are thought to have a pathogenic role in autoimmune and inflammatory diseases, including in granulomas. Here, we report that the PDZ-LIM domain protein PDLIM2 inhibited T_H17 cell development and granulomatous responses by acting as a nuclear ubiquitin E3 ligase that targeted signal transducer and activator of transcription 3 (STAT3), a transcription factor critical for the commitment of naïve CD4⁺ T cells to the T_H17 lineage. PDLIM2 promoted the polyubiquitination and proteasomal degradation of STAT3, thereby disrupting STAT3-mediated gene activation. Deficiency in PDLIM2 resulted in the accumulation of STAT3 in the nucleus, enhanced the extent of T_H17 cell differentiation, and exacerbated granuloma formation. This study delineates an essential role for PDLIM2 in inhibiting T_H17 cell-mediated inflammatory responses by suppressing STAT3 signaling and provides a potential therapeutic target for the treatment of autoimmune diseases.

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ERKs Induce Expression of the Transcriptional Repressor Blimp-1 and Subsequent Plasma Cell Differentiation

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In immune cells, the positive role of the extracellular signal-regulated kinase (ERK) signaling pathway in cell cycle progression and survival is well established; however, it is unclear whether ERK signaling plays a role in cell differentiation. Here, we report that ERKs are essential for the differentiation of B cells into antibody-producing plasma cells and that ERKs induce the expression of *Prdm1*, which encodes Blimp-1, a transcriptional repressor and “master regulator” of plasma cell differentiation. Transgenic mice with conditional deletion of both ERK1 and ERK2 in germinal center (GC) B cells lacked plasma cells differentiated after GC formation, and memory B cells from these mice failed to differentiate into plasma cells. In addition, ERK1- and ERK2-deficient B cells exhibited impaired *Prdm1* expression upon stimulation with antibody against CD40 in the presence of interleukin-4; conversely, enforced expression of *Prdm1* in ERK1- and ERK2-deficient B cells restored the generation of plasma cells. Thus, our study suggests that cytokines stimulate ERKs to induce the production of Blimp-1 and that ERKs thereby contribute to the process of cellular differentiation.

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Induction of Colonic Regulatory T Cells by Indigenous *Clostridium* Species

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CD4⁺ T regulatory cells (T_{regs}), which express the Foxp3 transcription factor, play a critical role in the maintenance of immune homeostasis. Here, we show that in mice, T_{regs} were most abundant in the colonic mucosa. The spore-forming component of indigenous intestinal microbiota, particularly clusters IV and XIVa of the genus *Clostridium*, promoted T_{regs} cell accumulation. Colonization of mice by a defined mix of *Clostridium* strains provided an environment rich in transforming growth factor- β and affected Foxp3⁺ T_{reg} number and function in the colon. Oral inoculation of *Clostridium* during the early life of conventionally reared mice resulted in resistance to colitis and systemic immunoglobulin E responses in adult mice, suggesting a new therapeutic approach to autoimmunity and allergy.

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Identification of Therapeutic Targets for Quiescent, Chemotherapy-Resistant Human Leukemia Stem Cells

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Human acute myeloid leukemia (AML) originates from rare leukemia stem cells (LSCs). Because these chemotherapy-resistant LSCs are thought to underlie disease relapse, effective therapeutic strategies specifically targeting these cells may be beneficial. Here, we report identification of a primary human LSC gene signature and functional characterization of human LSC-specific molecules in vivo in a mouse xenotransplantation model. In 32 of 61 (53%) patients with AML, either CD32 or CD25 or both were highly expressed in LSCs. CD32- or CD25-positive LSCs could initiate AML and were cell cycle-quiescent and chemotherapy-resistant in vivo. Normal human hematopoietic stem cells depleted of CD32- and CD25-positive cells maintained long-term multilineage hematopoietic reconstitution capacity in vivo, indicating the potential safety of treatments targeting these molecules. In addition to CD32 and CD25, quiescent LSCs within the bone marrow niche also expressed the transcription factor WT1 and the kinase HCK. These molecules are also promising targets for LSC-specific therapy.

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Preferential Generation of Follicular B Helper T Cells from Foxp3⁺ T Cells in Gut Peyer's Patches

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Most of the immunoglobulin A (IgA) in the gut is generated by B cells in the germinal centers of Peyer's patches through a process that requires the presence of CD4⁺ follicular B helper T (T_{FH}) cells. The nature of these T_{FH} cells in Peyer's patches has been elusive. Here, we demonstrate that suppressive Foxp3⁺CD4⁺ T cells can differentiate into T_{FH} cells in mouse Peyer's patches. The conversion of Foxp3⁺ T cells into T_{FH} cells requires the loss of Foxp3 expression and subsequent interaction with B cells. Thus, environmental cues present in gut Peyer's patches promote the selective differentiation of distinct helper T cell subsets, such as T_{FH} cells.

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A Critical Role for the Innate Immune Signaling Molecule IRAK-4 in T Cell Activation

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IRAK-4 is a protein kinase that is pivotal in mediating signals for innate immune responses. Here, we report that IRAK-4 signaling is also essential for eliciting adaptive immune responses. Thus, in the absence of IRAK-4, in vivo T cell responses were significantly impaired. Upon T cell receptor stimulation, IRAK-4 is recruited to T cell lipid rafts, where it induces downstream signals, including protein kinase C θ activation through the association with Zap70. This signaling pathway was found to be required for optimal activation of nuclear factor κ B. Our findings suggest that T cells use this critical regulator of innate immunity for the development of acquired immunity, suggesting that IRAK-4 may be involved in direct signal cross talk between the two systems.

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Autoimmune Disease and Impaired Uptake of Apoptotic Cells in MFG-E8–Deficient Mice

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Apoptotic cells expose phosphatidylserine and are swiftly engulfed by macrophages. Milk fat globule epidermal growth factor (EGF) factor 8 (MFG-E8) is a protein that binds to apoptotic cells by recognizing phosphatidylserine and that enhances the engulfment of apoptotic cells by macrophages. We report that tingible body macrophages in the germinal centers of the spleen and lymph nodes strongly express MFG-E8. Many apoptotic lymphocytes were found on the *MFG-E8*^{−/−} tingible body macrophages, but they were not efficiently engulfed. The *MFG-E8*^{−/−} mice developed splenomegaly, with the formation of numerous germinal centers, and suffered from glomerulonephritis as a result of autoantibody production. These data demonstrate that MFG-E8 has a critical role in removing apoptotic B cells in the germinal centers and that its failure can lead to autoimmune diseases.

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Innate Antiviral Responses by Means of TLR7-Mediated Recognition of Single-Stranded RNA

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Interferons (IFNs) are critical for protection from viral infection, but the pathways linking virus recognition to IFN induction remain poorly understood. Plasmacytoid dendritic cells produce vast amounts of IFN- α in response to the wild-type influenza virus. Here, we show that this requires endosomal recognition of influenza genomic RNA and signaling by means of Toll-like receptor 7 (TLR7) and MyD88. Single-stranded RNA (ssRNA) molecules of nonviral origin also induce TLR7-dependent production of inflammatory cytokines. These results identify ssRNA as a ligand for TLR7 and suggest that cells of the innate immune system sense endosomal ssRNA to detect infection by RNA viruses.

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Role of Adaptor TRIF in the MyD88-Independent Toll-Like Receptor Signaling Pathway

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Stimulation of Toll-like receptors (TLRs) triggers activation of a common MyD88-dependent signaling pathway as well as a MyD88-independent pathway that is unique to TLR3 and TLR4 signaling pathways leading to interferon (IFN)- β production. Here we disrupted the gene encoding a Toll/IL-1 receptor (TIR) domain-containing adaptor, TRIF. TRIF-deficient mice were defective in both TLR3- and TLR4-mediated expression of IFN- β and activation of IRF-3. Furthermore, inflammatory cytokine production in response to the TLR4 ligand, but not to other TLR ligands, was severely impaired in TRIF-deficient macrophages. Mice deficient in both MyD88 and TRIF showed complete loss of nuclear factor kappa B activation in response to TLR4 stimulation. These findings demonstrate that TRIF is essential for TLR3- and TLR4-mediated signaling pathways facilitating mammalian antiviral host defense.

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Control of Regulatory T Cell Development by the Transcription Factor *Foxp3*

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Regulatory T cells engage in the maintenance of immunological self-tolerance by actively suppressing self-reactive lymphocytes. Little is known, however, about the molecular mechanism of their development. Here we show that *Foxp3*, which encodes a transcription factor that is genetically defective in an autoimmune and inflammatory syndrome in humans and mice, is specifically expressed in naturally arising CD4⁺ regulatory T cells. Furthermore, retroviral gene transfer of *Foxp3* converts native T cells toward a regulatory T cell phenotype similar to that of naturally occurring CD4⁺ regulatory T cells. Thus, *Foxp3* is a key regulatory gene for the development of regulatory T cells.

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Critical Roles of Activation-Induced Cytidine Deaminase in the Homeostasis of Gut Flora

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Activation-induced cytidine deaminase (AID) plays an essential role in class switch recombination (CSR) and somatic hypermutation (SHM) of immunoglobulin genes. We report here that deficiency in AID results in the development of hyperplasia of isolated lymphoid follicles (ILFs) associated with a 100-fold expansion of anaerobic flora in the small intestine. Reduction of bacterial flora by antibiotic treatment of AID^{-/-} mice abolished ILF hyperplasia as well as the germinal center enlargement seen in secondary lymphoid tissues. Because an inability to switch to immunoglobulin A on its own does not lead to a similar phenotype, these results suggest that SHM of ILF B cells plays a critical role in regulating intestinal microflora.

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There's only one GALILEO GALILEI

Born in 1564, Galileo Galilei once contemplated a career in the priesthood. It's perhaps fortunate for science that upon the urging of his father, he instead decided to enroll at the University of Pisa. His career in science began with medicine and from there he subsequently went on to become a philosopher, physicist, mathematician, and astronomer, for which he is perhaps best known. His astronomical observations and subsequent improvements to telescopes built his reputation as a leading scientist of his time, but also led him to probe subject matter counter to prevailing dogma. His expressed views on the Earth's movement around the sun caused him to be declared suspect of heresy, which for some time led to a ban on the reprinting of his works.

Galileo's career changed science for all of us and he was without doubt a leading light in the scientific revolution, which is perhaps why Albert Einstein called him the father of modern science.

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